Chronic hypoxia-induced spontaneous and rhythmic contractions in the rat main pulmonary artery

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The molecular and cellular mechanisms underlying PAHT are not fully elucidated. Hypoxic PAHT is accompanied by an important remodeling of the pulmonary arterial wall, including cellular hypertrophy and hyperplasia, which decreases the lumen of the vessels and induces loss of elasticity of the arterial wall (5, 19, 25, 30). These changes also contribute to modification of pulmonary artery reactivity. The effect of CH on mediator-induced pulmonary artery contractility has already been examined, although variable modulation of reactivity has been reported, in particular depending on the type of mediator and on the considered portion of the pulmonary vascular bed (4, 17, 19, 20). In contrast, to the best of our knowledge, no detailed study has yet been performed to examine the effect of CH on intrinsic contractility (i.e., mechanical activity in the absence of exogenous stimulation). In normoxic conditions, pulmonary artery smooth muscle is electrically and mechanically quiescent. In the present study, we report that CH induces spontaneous and rhythmic contractions (SRCs) in the rat main pulmonary artery (MPA) and thus switches the MPA from a quiescent to an active status. The occurrence of SRCs has been previously briefly evoked in pulmonary vascular muscle exposed to normobaric hypoxia (1, 33) and, more extensively, in some vascular and visceral smooth muscles under normoxic conditions (9, 13–15). However, both the mechanism and role of such SRCs are not understood.

The present work was thus designed to characterize, in vitro, SRCs in the MPA from chronically hypoxic rats. Isometric contraction of the rings and measurement of the intracellular free Ca2+ concentration ([Ca2+]i) and membrane potential of smooth muscle cells with microspectrofluorometry (indo 1) and conventional micro-electrodes, respectively, were used to investigate the underlying mechanisms. Finally, the duration of hypoxic exposure was altered from 1 to 4 wk to examine the relationship between the time course of SRC occurrence and the development of right cardiac hypertrophy.

CHRONIC HYPOXIA (CH), which often occurs in patients suffering from pulmonary obstructive diseases (e.g., chronic bronchitis) (23, 26), secondarily induces a sustained elevation in pulmonary blood pressure. The subsequent pulmonary artery hypertension (PAHT) leads to right ventricular hypertrophy (RVH), right heart failure, and, ultimately, death (5, 25). This pathophysiologic adaptation of the pulmonary circulation is a complicated process for which no curative treatment is yet available.

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MATERIALS AND METHODS

CH. Adult male Wistar rats (aged 8–10 wk, 220–240 g) were separated into two groups. One group (control or normoxic rats) was housed in room air at a normal atmospheric pressure (101 kPa), and the other group (hypoxic rats) was kept in a hypobaric chamber for 1–4 wk, with most of the experiments being conducted after a 14-day exposure to CH. The pressure in the chamber was reduced to 0.5 atmosphere (50.5 kPa) with an electrically driven pump. The chamber was opened for 15–30 min twice a week. Pulmonary hypertension was assessed by measuring the ratio of right ventricle (RV) to left ventricle plus septum (LV+S) weight (2).

Tissue and cell preparation. The rats were anesthetized by intraperitoneal administration of 400 mg of ethyl carbamate. The MPA was dissected, and the adventitial layer was removed. For contraction and electrophysiological experiments, rings (3 mm in length) were dissected in a Krebs-Henseleit solution (composition in mM: 118.4 NaCl, 4.7 KCl, 2.5 CaCl₂·2H₂O, 1.2 MgSO₄·7H₂O, 1.2 KH₂PO₄, 25.0 NaHCO₃, and 11.1 D-glucose, pH 7.4) maintained at 37°C and bubbled with a 95% O₂-5% CO₂ gas mixture. For [Ca²⁺]ᵢ measurements, enzymatically isolated vascular myocytes were obtained with procedures previously described (12, 16).

Fig. 1. Effect of normoxia (Aa, Ba, and Ca) and chronic hypoxia (CH; Ab, Bb, and Cb) on both mechanical (tension; A) and electrical (membrane potential; B) activities of main pulmonary artery (MPA) rings and intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ; C) of isolated MPA myocytes. Rats were exposed for 14 days to CH. Note spontaneous contractions (Ab) and depolarizations (Bb) induced by CH.

Table 1. Effect of drugs on amplitude and frequency of SRC and tone of pulmonary arterial rings from hypoxic rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>Amplitude, mg</th>
<th>Frequency, min⁻¹</th>
<th>Tone, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>136 ± 93</td>
<td>7.6 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>+Tetrodotoxin</td>
<td>8</td>
<td>132 ± 95</td>
<td>7.4 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>227 ± 184</td>
<td>6.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>+Phentolamine</td>
<td>16</td>
<td>242 ± 178</td>
<td>6.4 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>262 ± 168.7</td>
<td>4 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>+Atropine</td>
<td>8</td>
<td>241 ± 179.4</td>
<td>3.75 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>145 ± 47.5</td>
<td>7.5 ± 1</td>
<td></td>
</tr>
<tr>
<td>+l-NAME</td>
<td>8</td>
<td>136.25 ± 46.9</td>
<td>7.25 ± 1.4</td>
<td>275 ± 91.6</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>222 ± 62.4</td>
<td>5.2 ± 0.96</td>
<td></td>
</tr>
<tr>
<td>+BQ-123 and BQ-788</td>
<td>10</td>
<td>388 ± 65.6</td>
<td>4.4 ± 0.9</td>
<td>-560 ± 128</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of pulmonary arterial rings. SRC, spontaneous and rhythmic contraction; l-NAME, N-nitro-l-arginine methyl ester.
cells were maintained in a physiological salt solution (composition in mM: 130 NaCl, 5.6 KCl, 1 MgCl₂, 2 CaCl₂, 11 D-glucose, and 10 HEPES, pH 7.4 with NaOH) on glass coverslips at 4°C and used on the same day.

**Isometric contraction measurement.** Isometric contraction was measured in MPA rings in vertical 20-ml organ baths of a computerized isolated organ bath system (IOX, EMKA Technologies) previously described (2). The baths were filled with Krebs-Henseleit solution. As determined in control experiments, the rings obtained from control and CH rats were set at optimal length by equilibration of a passive load of 1 and 2 g, respectively. In some experiments, the endothelium of the ring was removed by gently rubbing the luminal surface with a cotton swab. Successful removal of the endothelium was confirmed by the inability of acetylcholine (1 μM) to induce 10% of relaxation in phenylephrine (1 μM)-contracted rings.

**[Ca²⁺]i measurement.** Changes in [Ca²⁺], of pulmonary artery myocytes were monitored fluorometrically with the Ca²⁺-sensitive probe indo 1 as previously described (12, 16). The recording system included a Nikon Diaphot inverted microscope fitted with epifluorescence (Nikon). Signals at each wavelength were digitized and stored on an IBM personal computer with a PC-Lab Card 812PG interface. A single cell selected from among those on the coverslip was tested through a window that was manually adjusted in each experiment to be slightly larger than the cell. The studied cell was illuminated at 360 nm and counted simultaneously at 405 and 480 nm by two photomultipliers (P100, Nikon, Tokyo, Japan). The fluorescence ratio (405- to 480-nm fluorescence) was calculated on-line and displayed with the two voltage signals on a monitor. [Ca²⁺], was estimated from the fluorescence ratio (11), with a calibration for indo 1 determined within pulmonary smooth muscle myocytes as previously described (12).

**Membrane potential measurement.** Membrane potential was measured in MPA rings with conventional microelectrodes. The microelectrode was inserted into the media from the luminal side by means of a hole previously made in the ring. Microelectrodes were filled with 3 M KCl, yielding a tip resistance of 40–80 MΩ, and were held by means of a micro-manipulator (Narishige). The microelectrode was connected...
Fig. 3. Effect of Ca$^{2+}$-free solution on SRCs of MPA rings and [Ca$^{2+}$]i, of isolated MPA myocytes from chronically hypoxic rats. A: removal of external Ca$^{2+}$ (a, arrow) rapidly suppressed SRC and tone, both of which reappeared after the reintroduction of Ca$^{2+}$ (b). B: original traces of [Ca$^{2+}$]i measurement in the presence (a) and 15 min after removal (b) of external Ca$^{2+}$. Inset: mean [Ca$^{2+}$]i, value before (open bar), during removal (solid bar), and after readdition (cross-hatched bar) of external Ca$^{2+}$. *P < 0.05.

RESULTS

Effect of CH on RVH and on contractility, membrane potential, and [Ca$^{2+}$]i of the MPA. In rats exposed to hypobaric hypoxia for 14 days, the ratio of RV to LV+S weight was significantly increased compared with that in control rats [0.48 ± 0.11 (n = 17) and 0.29 ± 0.02 (n = 10), respectively; P < 0.05].

MPA rings from CH rats exhibited SRCs that were superimposed on a basal tone (Fig. 1Ab). The amplitude and frequency of these contractions were 238.7 ± 112.2 mg [i.e., 22.9% of K$^{+}$-rich (80 mM) solution-induced contraction] and 6.5 ± 1.2 min$^{-1}$ (n = 35), respectively. These contractions remained constant and could be observed for as long as 8–10 h in control conditions. In contrast, SRCs and tone were absent in MPA rings from normoxic rats (n = 12; Fig. 1Aa). Moreover, SRCs were never observed when MPA rings from normoxic rats were equilibrated against a load of 2 g, i.e., the load that stretches MPA rings from hypoxic rats to optimal length (data not shown). Regarding the site along the pulmonary vascular bed, SRCs appeared in 90% (n = 20) of the rings obtained from MPAs but only in 37.5 (n = 32) and 6.7% (n = 12) of the rings obtained from the second branches of the PA and from intrapulmonary arteries (IPA), respectively (data not shown).

CH-induced changes in contractility were accompanied by changes in the electrical properties of pulmonary arterial rings. The resting membrane potential was significantly altered (−38.6 ± 5.4 and −58.8 ± 9 mV in rings from CH and normoxic rats, respectively; P < 0.05; n = 3; Fig. 1B). Furthermore, although the membrane potential was stable in rings from normoxic rats, large spontaneous depolarizations (21.3 ± 7.4 mV; n = 3) similar to action potentials were observed in rings from CH rats (Fig. 1B). The frequency of those spontaneous depolarizations was similar to that of the SRCs, i.e., 6.1 ± 1.5 min$^{-1}$ (n = 3).
Finally, the resting $[\text{Ca}^{2+}]_i$ value was significantly higher in arterial myocytes from CH rats compared with normoxic rats (112.5 ± 16.4 and 67.3 ± 11.9 nM, respectively; $P < 0.05$; $n = 270$; Fig. 1C).

Involvement of nervous and endothelium-derived factors in SRCs from CH rats. The Na$^+$ channel antagonist tetrodotoxin (0.1–1 μM) inhibited neither the basal tone nor the SRC. The amplitude and frequency of SRCs remained unchanged (Table 1, Fig. 2A). A similar result was obtained in the presence of phentolamine (1 μM; $n = 16$), an α-adrenoceptor antagonist, and atropine (10 μM; $n = 8$), a cholinergic receptor antagonist (Table 1). L-NAME (10 μM), a competitive inhibitor of nitric oxide synthase (21), increased the basal tone but did not significantly alter the SRC ($n = 8$; Fig. 2Bb, Table 1). The addition of a combination of BQ-123 and BQ-788 (1 μM each), selective antagonists of type A and type B endothelin-1 receptors, respectively (7), decreased the basal tone without altering the SRC ($n = 10$) (Table 1, Fig. 2Bd). Finally, endothelium-denuded MPA rings exhibited SRCs ($n = 9$) with the same amplitude and frequency as those from intact MPA rings ($n = 7$; Fig. 2C).

Effect of external Ca$^{2+}$ removal and Ca$^{2+}$ channel antagonists on spontaneous contractions and depolarizations in rings from CH rats. Superfusion of MPA rings with Ca$^{2+}$-free Krebs solution containing 0.4 mM EGTA caused a rapid inhibition of SRCs accompanied by a relaxation ($n = 8$). This effect was rapidly reversible, i.e., 2–5 min after Ca$^{2+}$ was readded into the Krebs solution, SRCs reappeared, and the basal tone progressively reached its control level (Fig. 3A). Superfusion of myocytes with a Ca$^{2+}$-free solution also decreased resting $[\text{Ca}^{2+}]_i$ value from 115 ± 12 to 70 ± 9 nM ($P < 0.05$; $n = 90$; Fig. 3B). Reintroduction of Ca$^{2+}$ into the extracellular solution restored the resting $[\text{Ca}^{2+}]_i$ value (103 ± 10 nM; $n = 90$). In contrast, removal of the external Ca$^{2+}$ did not alter $[\text{Ca}^{2+}]_i$ in myocytes from normoxic rats ($n = 30$).

Nifedipine (1 μM), a voltage-dependent Ca$^{2+}$ channel antagonist (31), had similar effects to those induced by Ca$^{2+}$ removal (Fig. 4). However, recovery of SRC and...
basal tone was more delayed than after Ca\(^{2+}\) readmission and required 20–30 min after washout of the drug. The pretreatment of myocytes from CH rats with D-600 (10 \(\mu\)M), a voltage-dependent Ca\(^{2+}\) channel antagonist (31), for 15 min significantly decreased the resting [Ca\(^{2+}\)]\(_i\) value from 120 ± 10 to 80 ± 8 nM (\(P < 0.05; n = 90; \text{Fig. 4B}\)). In contrast, D-600 (10 \(\mu\)M) did not alter the [Ca\(^{2+}\)]\(_i\) in myocytes from normoxic rats (\(n = 30\)). Finally, nifedipine (1 \(\mu\)M) did not alter the mean value of the resting membrane potential (−34.2 ± 1.1 and −37.2 ± 2 mV in the absence and presence, respectively, of nifedipine; \(n = 3\)) but reduced spontaneous depolarizations (Fig. 4C). Similar results were obtained with D-600 (10 \(\mu\)M; \(n = 3\); data not shown).

**Effect of membrane potential modulation on SRCs.**

In the presence of levcromakalim (0.1–1 \(\mu\)M), a K\(^+\) channel opener (6), SRCs disappeared, and the basal tone fell (Fig. 5A). The pretreatment of MPA myocytes from CH rats with levcromakalim for 15–20 min decreased the resting [Ca\(^{2+}\)]\(_i\) value from 117 ± 16 to 90 ± 16 nM (\(P < 0.05; n = 90; \text{Fig. 5B}\)). Finally, as expected, the addition of levcromakalim (0.1 \(\mu\)M) repolarized the smooth muscle membrane from −34.4 ± 4.8 to −50.2 ± 4.8 mV (\(P < 0.05; n = 3\); Fig. 5C) and abolished the spontaneous depolarizations. Similar experiments with levcromakalim were conducted on pulmonary arteries from normoxic rats, and no effect on either tone (\(n = 8\)), [Ca\(^{2+}\)]\(_i\) (\(n = 30\)), or membrane potential (\(n = 3\)) was observed (data not shown). In another set of experiments, we tested the effect of 4-aminopyridine (4-AP), a K\(^+\) channel antagonist (6). Low concentrations (100 \(\mu\)M to 1 mM) of 4-AP increased the amplitude of SRCs concentration dependently, whereas in the presence of high (1–20 mM) 4-AP concentrations, the SRCs disappeared together with a large increase in the basal tone (Fig. 6A). Concentration-response curves reveal
that both reactivity to and potency for 4-AP were increased in rings from CH rats compared with rings from normoxic rats (Fig. 6B). Finally, we examined the effect of ChTX (20–200 nM), a specific blocker of Ca\(^{2+}\)-activated K\(^{+}\) channels (6). ChTX had no effect on the SRCs (n = 8; data not shown).

**Involvement of intracellular Ca\(^{2+}\) and Ca\(^{2+}\)-induced Ca\(^{2+}\) release in SRCs.** CPA, a sarcoplasmic reticulum Ca\(^{2+}\)-ATPase inhibitor (10, 28), and ryanodine, the activator of the so-called ryanodine receptor Ca\(^{2+}\) channel (35), were used at low concentrations to slowly deplete intracellular Ca\(^{2+}\) stores without inducing contraction. The pretreatment of rings with CPA (0.1–3 μM) in the presence of L-NAME (10 μM) suppressed SRCs according to both time and concentration and also decreased basal tone (n = 10; Fig. 7A). Ryanodine (1–5 μM) progressively inhibited SRCs and basal tone (n = 18; Fig. 7B). This inhibitory effect of ryanodine was maintained in the presence of ChTX (200 nM; n = 10; data not shown).

Because SRCs appeared to be dependent on both extracellular and intracellular Ca\(^{2+}\) sources, we examined the possible involvement of a Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) mechanism using tetracaine (35). Tetracaine (50–100 μM), which had no effect on K\(^{+}\)-rich (80 mM) solution, progressively abolished SRCs (n = 6; Fig. 7C).

**Involvement of gap junctions in SRCs.** The role of gap junctions in the generation of SRCs was examined with the putative gap junction uncoupling agent 18\(^{\alpha}\)-glycyrrhetinic acid (3). In the presence of 18\(^{\alpha}\)-glycyrrhetinic acid (20–40 μM), SRCs rapidly (1–2 min) vanished and basal tone concomitantly decreased (n = 8; Fig. 8). These effects were fully reversed within 2–5 min on washout of the drug.

**Time course of SRCs and RVH development.** In this final series of experiments, we examined the effect of altering the duration of the hypoxic exposure (1–4 wk) on both the occurrence of SRCs in the MPA and the severity of RVH. Figure 9A indicates that the higher the severity of RVH (as assessed by the RV-to-LV+S ratio), the lower the occurrence of SRCs; i.e., the development of SRCs preceded that of RVH. We paid special attention to the situation after 1 wk of CH because it corresponds to the onset of RVH development. As expected, at that stage, the severity of RVH and the amplitude of SRCs were scattered. However, there was...
an inverse relationship between both factors; i.e., the higher the SRC amplitude, the lower the severity of RVH (Fig. 9B). It is noteworthy that the disappearance of SRCs was not accompanied by repolarization of pulmonary vascular smooth muscle cells because the mean membrane potential values were $-33.7 \pm 3.4$ ($n=11$) and $-30.1 \pm 1.8$ ($n=9$) mV after 3 and 4 wk of CH exposure, respectively. These results suggest that alternative mechanisms account for the disappearance of SRCs in depolarized pulmonary arteries.

**DISCUSSION**

The main result of the present study is that 14 days of CH switches the rat MPA from a quiescent to a spontaneously active mechanical state. CH-induced SRCs coincide with alterations in both resting $[Ca^{2+}]_i$ value and electrical activity of pulmonary vascular smooth muscle cells. The fact that SRCs precede the development of RVH suggests that they may play a role in the adaptive process of the pulmonary circulation to CH.

In the rat MPA, CH induced SRCs, with a concomitant increase in basal tone. Removal of external Ca$^{2+}$ as well as the addition of the majority of drugs that inhibited SRCs also decreased basal tone and $[Ca^{2+}]_i$ in myocytes. These results suggest a direct relationship between SRCs and the development of tone caused by an increase in $[Ca^{2+}]_i$. That is, SRCs were never observed in MPA rings from control (normoxic) rats even when the resting tension was similar to that applied to MPA rings from CH rats (e.g., 2 g), demonstrating that SRCs and tone occurrence are independent of the resting tension.

CH-induced SRCs did not depend on the release of neurotransmitters from nerve endings because they were not altered in the presence of tetrodotoxin, phenolamine, or atropine. Also, CH-induced SRCs did not depend directly on an endothelium-derived factor. We paid special attention to endothelin-1 because it is well established that it is increased in the pulmonary circulation from both rats and humans with PAHT (1, 8, 18). The endothelin-1 receptor antagonists BQ-123 and BQ-788 did not alter SRC amplitude, although they decreased basal tone. L-NAME also had no effect on SRCs but increased basal tone, which thus appears to be modulated by endogenous release from the endothelium, negatively by nitric oxide and positively by endothelin-1. Finally, no significant difference was observed in the spontaneous activity between intact and endothelium-denuded rings. Collectively, these data demonstrate that SRCs in the MPA from CH rats are myogenic.

Removal of external Ca$^{2+}$ or addition of L-type Ca$^{2+}$ channel antagonists rapidly abolished SRCs, demonstrating the involvement of Ca$^{2+}$ influx via L-type Ca$^{2+}$ channels. L-type Ca$^{2+}$ channels require membrane depolarization to operate. In the present study, we confirm the depolarizing effect of CH ($-20$ mV) in MPA smooth muscle as previously observed in isolated cells from IPAs of both hypoxic rats (22, 29) and humans with primary pulmonary hypertension (34). A less large depolarization has also been shown in intact rings from small IPAs (24). The difference in the amplitude of the depolarization between this observation and the present results may be related to the site along the vascular bed (MPA vs. IPA), which would also explain why we observed fewer SRCs in distal arteries.

**Fig. 9.** Effect of the duration of the hypoxic exposure (1–4 wk) on both the occurrence of SRCs in MPA and the severity of right ventricular hypertrophy (RVH). A: occurrence of SRCs as assessed by the percentage of rings exhibiting SRCs and severity of RVH assessed as in A in rats after 1 wk of hypoxia.
There is evidence that the CH-induced membrane depolarization is related to alterations in K+ channel activity. This depolarization has been ascribed to a downregulation of voltage-gated K+ channels (Kv1.2 and Kv1.5) (32) switching the major K+ current determining the membrane potential from a noninactivating K+ current to the 4-AP-sensitive voltage-gated K+ current (22). The fact that SRCs and the tone of rings from CH rats are very sensitive to 4-AP (Fig. 6) further supports this hypothesis in the MPA. A main participation of K+ channels in the CH-induced depolarization is also supported by the absence of an effect of nifedipine and D-600 on the resting membrane potential (Fig. 4). These findings thus exclude a contribution of L-type Ca2+ channels in this depolarization.

For the first time to our knowledge, we have observed that in MPAs from CH rats spikelike spontaneous depolarizations and SRCs disappeared in the absence of external Ca2+. This similarity strongly suggests a link between the two phenomena; i.e., the development of SRCs is likely to be controlled by membrane potential variations. In this connection, the fact that levocromakalim, which hyperpolarizes the myocyte, abolished both SRCs and spontaneous and rhythmic depolarizations (Fig. 5) supports this hypothesis. It is noteworthy that levocromakalim repolarized MPA smooth muscle from hypoxic rats to a membrane potential value similar to that of smooth muscle from normoxic rats in which SRCs were absent.

Drugs acting at the sarcoplasmic reticulum, CPA and ryanodine, progressively abolished SRCs, suggesting a key role for intracellular Ca2+ stores in the mechanism of SRCs. The link between Ca2+ influx and Ca2+ release could be a CICR mechanism. This hypothesis is supported by the inhibitory effect of tetracaine, a CICR blocker (35), on SRCs at concentrations that did not block Ca2+ channels as demonstrated by the persistence of K+-rich solution-induced contraction.

In our study, although CH induced both spontaneous Ca2+-dependent contractions and depolarizations, we did not observe spontaneous variations in [Ca2+]i. Several technical and methodological factors could account for this apparent discrepancy between both mechanical and electrical activities and intracellular Ca2+ measurements. First, although the former activities were recorded in multicellular preparations, [Ca2+]i was measured in single cells. Spontaneous activity could result from the functioning of a syncytium that is likely to be the case in PAHT due to cell phenotype change induced by CH (30). SRCs thus may require the presence of several cells coupled by gap junctions. The rapid inhibition of SRCs caused by 18α-glycyrrhetinic acid (Fig. 8) argues in favor of this hypothesis. Similar inhibition of both spontaneous electrical and mechanical activities by gap junction uncoupling agents has also been observed in other smooth muscles under normoxic conditions (14, 27). This set of data is in agreement with the more global concept of a CH-induced switch from a quiescent (or multiunit) to a spontaneously active (or single-unit) pulmonary smooth muscle. Second, because our data suggest the involvement of Ca2+ release from the intracellular store, it is possible that this release operates in a localized space close to the contractile apparatus. Such a localized phenomenon is rather difficult to detect with whole cell microspectrofluorometry, and confocal microscopy would be necessary to address this issue.

SRCs occurred in preparations taken from animals with 14 days of severe hypoxia, a condition known to induce both remodeling of the pulmonary artery and PAHT (4, 17, 19, 25). In a first attempt to understand the role of SRCs, we examined the time course of their occurrence. When the duration of hypoxic exposure was altered from 1 to 4 wk, the occurrence of SRCs diminished with the duration of CH along with an increase in RVH severity. Thus SRCs precede the development of RVH and disappear when RVH is maximum. Moreover, after 1 wk of CH, at the onset of the development of RVH when individual variability is important, the higher the amplitude of SRCs, the lower the severity of RVH. This relationship suggests that SRCs may represent a temporary adaptive process of the pulmonary circulation to CH.

In conclusion, the present study shows that SRCs accompany PAHT induced by 14 days of hypobaric CH. The mechanism of SRCs is mainly dependent on a depolarization of pulmonary myocytes triggering spontaneous Ca2+ spikelike potential via activation of L-type Ca2+ channels. A CICR mechanism seems also to be involved. Finally, the fact that SRCs precede the development of RVH and disappear when this hypertrophy reaches a maximal value (after 3–4 wk of CH) suggests that SRCs may play a hemodynamic role during this transient phase that stops when RVH is constituted.

The time course of the development of SRCs suggests that they may represent a temporary adaptive process of the pulmonary circulation in the course of hypoxia-induced PAHT and RVH.

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REFERENCES