T-type calcium channels are involved in hypoxic pulmonary hypertension

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Pulmonary hypertension (PH) is the main disease of pulmonary circulation. Alteration in calcium homeostasis in pulmonary artery smooth muscle cells (PASMCs) is recognized as a key feature in PH. The present study was undertaken to investigate the involvement of T-type voltage-gated calcium channels (T-VGCCs) in the control of the pulmonary vascular tone and thereby in the development of PH.

Methods and results
Experiments were conducted in animals (rats and mice) kept 3–4 weeks in either normal (normoxic) or hypoxic environment (hypobaric chamber) to induce chronic hypoxia (CH) PH. In vivo, chronic treatment of CH rats with the T-VGCC blocker, TTA-A2, prevented PH and the associated vascular hyperreactivity, pulmonary arterial remodelling, and right cardiac hypertrophy. Deletion of the Ca3.1 gene (a T-VGCC isoform) protected mice from CH-PH. In vitro, patch-clamp and PCR experiments revealed the presence of T-VGCCs (mainly Ca3.1 and Ca3.2) in PASMCs. Mibebradil, NNIC550396, and TTA-A2 inhibited, in a concentration-dependent manner, T-VGCC current, KCl-induced contraction, and PASMC proliferation.

Conclusion
The present study demonstrates that T-VGCCs contribute to intrapulmonary vascular reactivity and is implicated in the development of hypoxic PH. Specific blockers of T-VGCCs may thus prove useful for the therapeutic management of PH.

Keywords
Arterial reactivity • Calcium channel blockers • Pulmonary circulation • Patch-clamp • T-type calcium currents

1. Introduction
Pulmonary circulation is a specific circulation with low pressure and high flow. Pulmonary hypertension (PH) is the main disease of the pulmonary circulation. According to the classification of PH, pulmonary arterial hypertension, the most evident form of PH, is a fatal disease characterized by an increase in pulmonary blood pressure (mean value >25 mmHg) and vascular resistance. It leads to a right ventricular heart failure and ultimately to death.1 This disease is associated with hyperreactivity to vasoconstrictor agents such as serotonin2 and a remodelling of pulmonary arteries with proliferation and migration of pulmonary arterial smooth muscle cells (PASMCs).3,4

An increase in the intracellular calcium concentration ([Ca2+]i) is a critical event involved in PASMC contraction, proliferation, and migration which evolves during PH.4 Evidence indicates that changes in the expression and function of ionic channels involved in [Ca2+]i handling such as potassium channels, voltage-independent channels (TRPC1), or voltage-gated calcium channels (VGCCs) are implicated in PH.5–7 VGCCs constitute a main route for calcium influx into PASMCs.8 The family of VGCCs is divided into high-voltage-gated channels (L-VGCCs, for instance, in PASMC) and low-voltage-gated T-type channels (T-VGCCs). Three isotypes (Ca3.1, Ca3.2, and Ca3.3) encode for T-VGCC. Although L- and T-VGCCs are both activated by depolarization, L-VGCCs are mainly activated by large depolarization, whereas T-VGCCs are activated near to the resting membrane potential and inactivate rapidly.7

Although L-VGCC inhibitors are largely used in the treatment of systemic vascular diseases, they are not so efficient in the treatment of PH.10 We hypothesize that T-VGCC could constitute an alternative therapeutic target in PH, since they also participate in cell proliferation and in the vascular tone of pulmonary arteries. Indeed, T-VGCC protein expression is increased in PH11,12 and dehydroepiandrosterone (DHEA), a T-VGCC inhibitor,13 prevents PH.14 Those channels are...
involved in vascular contraction induced by N omega-nitro-L-arginine (L-NNa) in rat hypertensive pulmonary arteries \textsuperscript{14} or by serotonin in bovine pulmonary vessels. \textsuperscript{15} Finally, the selective blockade of Ca\textsubscript{3.1} expression (a T-VGCC isoform) inhibits PASMC proliferation, \textit{in vitro}. \textsuperscript{16,17} Studies on T-VGCCs have been hampered, thus far, by the use of weakly selective inhibitors (mibebradil, nickel, flunarizine, or pimozide). \textsuperscript{9,18} Whereas these drugs also target L-VGCC, new classes of blockers (TTA-A2 or NNC550396) are reported to be more selective \textsuperscript{19,20} and have therefore been used in this study.

The purpose of this study was thus to specifically evaluate the involvement of T-VGCC in pulmonary vascular reactivity and in PH. Using animal models of PH (chronically hypoxic rats and mice), genetically modified mice (Ca\textsubscript{3.1}\textsuperscript{+/-} mice), and selective T-VGCC blockers, we demonstrate that T-VGCCs are involved in the development of PH.

2. Methods

The complete section is provided in Supplementary material online, Data S1.

2.1 Animal models

The investigations were carried out according to the care and use of our local ethic committee (Comité d’Éthique de Bordeaux en Experimentation Animale N’50).

Wistar male rats (200–300 g) were housed in ambient room air (normoxic rats—N\textsubscript{x}) or in a hypobaric chamber (50 kPa) for 3 weeks to induce PH (chronic hypoxic rats—CH). At completion of the 3-week period, rats were anaesthetized with 10 mg/kg of xylazine and 50 mg/kg of ketamine by intraperitoneal injection, and mean right ventricle pressure (RVP), mice were anaesthetized with 10 mg/kg of pentobarbital, intraperitoneal injection. Such a protocol led to a TTA-A2 concentration in blood \textsim 1 \mu M.

2.7 TTA-A2 infusion in rats

The T-VGCC blocker, TTA-A2, was dissolved in vehicle: 10% ethanol, 40% PEG 400, and 25% hydroxypropyl-beta-cyclodextrin (10 mg/mL). TTA-A2 was delivered at the rate of 0.05 mg/h for 3 weeks using an osmotic mini-pump (Alzet, model 2ML4) implanted subcutaneously 2 days before exposition to CH (anaesthesia: 60 mg/kg pentobarbital, intraperitoneal injection). Such a protocol led to a TTA-A2 concentration in blood \textsim 1 \mu M.

2.8 Drugs

All drugs were from Sigma. TTA-A2 was provided by Merck and Co., Inc.

2.9 Statistical analysis

Data are given as mean \pm SEM of \textit{n} experiments. In Figures 5 and 6, \textit{n} was the number of animals tested. Statistical analyses were performed with a two-way ANOVA test for repeated measures (Figures 1C, 4A–C, and 6A), one-way ANOVA test with a Bonferroni correction for post-test (Figures 2A, B, D, and E, 3A, B, and D, 4D, 5D, and 6B and 6C), and Mann–Whitney test (Figure 5A). Differences were considered statistically significant when \textit{P} \textless 0.05.

3. Results

3.1 T-VGCCs are expressed in PASMCs

Depolarization of freshly isolated PASMCs from N\textsubscript{x} or CH rats activated a fast inward and transient current (Figure 1A and 8) in the presence of L-VGCC inhibitors. This current has similar properties to T-VGCC: (i) it inactivated rapidly (\textit{r} inactivation at \textsim 30 mV: 22 \pm 4 ms \textit{n} = 7 and 25 \pm 6 ms \textit{n} = 5, for N\textsubscript{x} and CH rats, respectively), (ii) the threshold of activation was around \textsim 50 mV, and (iii) the maximum peak current was measured around \textsim 30 mV (Figure 1C). Furthermore, T-VGCC blockers TTA-A2 (1 \mu M) and NNC550396 (1 \mu M) inhibited this current in N\textsubscript{x}-rats at all depolarizing potentials tested (Figure 1A and see Supplementary material online, Figure S2A–C). Ni\textsuperscript{2+} (50 \mu M) partially inhibited this current (\textsim 38 \pm 5\% \textit{n} = 3). The electrophysiological identification of T-VGCC current was also confirmed by analysis of RT-PCR using specific primers for Ca\textsubscript{3.1} and Ca\textsubscript{3.2} on RNA extracts from the media of rat IPA (Figure 1D). PASMC also expressed L-VGCC current. This current was completely inhibited by the L-VGCC antagonist diltiazem (see Supplementary material online, Figure S2D), partially inhibited by mibebradil (1 \mu M, 34\% inhibition, \textit{n} = 4), and NNC550396 (1 \mu M, 22\% inhibition, \textit{n} = 4), but almost not modified by TTA-A2 (1 \mu M, 4\% inhibition, \textit{n} = 4) (Figure 1E and F).
3.2 TTA-A2 prevents CH-induced PH

Since PASMCs express T-VGCCs, we investigated the involvement of T-VGCC in the development of PH by using TTA-A2 treatment in vivo. Rats were implanted with an osmotic pump filled with TTA-A2 and then kept in the hypobaric chamber for 3 weeks. Treatment with TTA-A2 prevented a CH-induced increase in both mean pulmonary pressure (37 and 25 mmHg, \( P < 0.05 \), respectively) and Fulton’s index (0.5 and 0.36, \( P < 0.05 \), respectively; Figure 2A and 2B). Treatment with TTA-A2 also protected from CH-induced wall thickness hypertrophy as shown in Figure 2C and D. We investigated whether the in vivo chronic treatment with TTA-A2 also prevented hyperreactivity of IPA to vasoconstrictors (KCl and serotonin). In vitro, 15 mM KCl induced a maximal contraction of 29 ± 4% (n = 7) (80 mM KCl) in IPA from CH rats compared with 5 ± 3% (n = 7) in IPA from Nx-rats. When CH rats were treated in vivo with TTA-A2, 15 mM KCl-induced contraction was reduced to 9 ± 3% (n = 7, \( P < 0.05 \)), showing that TTA-A2 prevented vascular hyperreactivity (Figure 2E). Moreover, chronic TTA-A2 treatment in vivo also reduced the hyperresponsiveness of CH-IPA to serotonin (Figure 2E).

3.3 Deletion of the Cav3.1 gene protected mice from PH

To further confirm the contribution of T-VGCC in the development of CH-induced PH, we performed experiments on Ca v3.1 null mice (Cav3.1\(^{-/-}\)). In Nx mice, RVP and Fulton’s index were not statically different between wild-type (WT) and Cav3.1\(^{-/-}\) mice (Figure 3). In WT mice, CH induced PH, confirmed by an increase in both RVP and Fulton’s index (Figure 3A and B). In contrast, no significant increase was observed in Cav3.1\(^{-/-}\) mice for any of these parameters (Figure 3A and B). Using morphological analysis, to compare CH-induced IPA remodelling in WT and Cav3.1\(^{-/-}\) mice, we observed that CH induced a potent remodelling in WT, but not in Cav3.1\(^{-/-}\) mice (Figure 3C and D). Taken together, these results show, for the first time, that the Cav3.1 contributes to the development of CH-induced PH.

3.4 T-VGCC and vascular reactivity in Nx-IPA and CH-IPA

To better understand the mechanisms by which T-VGCC blockers prevent PH, we investigated the effect of acute application of
3.4.1 T-VGCC blockers reduce KCl-induced contraction in IPA from Nx-rats

A non-cumulative concentration–response curve for KCl (15–80 mM, see Supplementary material online, Figure S1) was used to allow PASMC repolarization between each KCl application and to avoid T-VGCC inactivation. TTA-A2 (1, 10, or 30 μM) reduced the amplitude of KCl-induced contraction, in a concentration-dependent manner (Figure 4A). Moreover, its inhibitory effect was more pronounced for low than for high KCl concentrations: TTA-A2 (30 μM) reduced by 79% the 30 mM KCl-induced contraction and by only 35% the 80 mM KCl-induced contraction (Figure 4A and D). NNC550396 was also a powerful inhibitor of KCl-induced contraction with similar characteristics: at 10 μM, it fully abolished the 30 mM KCl-induced contraction and by only 35% the 80 mM KCl-induced contraction (Figure 4A and D). Mibefradil as well as flunarizine and pimozide inhibited KCl-induced contraction, but displayed the same inhibitory effect on contraction induced by either low (30 mM) or high (80 mM) KCl concentrations (Figure 4C and see Supplementary material online, Figure S3), which may be associated with an effect on both T- and L-VGCC. Finally, Ni²⁺ (50 μM) weakly inhibited the 30 mM KCl-induced contraction (14 ± 5%, n = 3, see Supplementary material online, Figure S3).

Since activation of T-VGCC depends on the resting membrane potential value, subsequent experiments were performed in the presence of cronicakalim, a well-known K-ATP channel opener, which hyperpolarizes PASMC.23 TTA-A2 and mibefradil were more powerful inhibitors of KCl-induced contraction in the presence of 10 μM cronicakalim (Figure 5A). Additionally, we performed a cumulative concentration–response curve to KCl, which favoured progressive cell depolarization and T-VGCC inactivation (see Supplementary material online, Figure S1). In these conditions, TTA-A2 (10 μM) had a smaller inhibitory effect on the 30 mM KCl-induced contraction than in the protocol performed in Figure 3 (26 ± 4%, n = 4, Figure 4 and 57 ± 6%, n = 8, Figure 5B).

T-VGCC blockers were tested in the presence of the L-VGCC blocker diltiazem. Diltiazem (5 μM), alone, decreased the contraction induced by non-cumulative doses of KCl (15–80 mM). However, TTA-A2 exhibited an additional inhibitory effect, mainly for low KCl concentrations (Figure 5C). NNC550396 had a similar effect on 40 mM KCl-induced contraction (Figure 5D).

Altogether, these results imply that, in Nx-rat IPA, KCl-induced contraction is sensitive to T-VGCC blockers, especially under conditions for which T-VGCC may be active (i.e. hyperpolarized resting membrane potential).
3.4.2 T-VGCC blockers reduce KCl-induced contraction in IPA from CH-rats

We observed that low KCl concentrations (15–40 mM) induced a greater contraction in CH-IPA than in Nx-IPA, indicating a hyperactivity of CH-IPA to a weak depolarization (Figure 6A and B). All T-VGCC blockers inhibited KCl-induced contraction on CH-IPA. The effect was similar to or slightly smaller than for Nx-rats (Figure 6B). As for Nx-rats, inhibitory effect of TTA-A2 was more pronounced for low than for high KCl concentrations: at 30 mM, TTA-A2 reduced by 85 and 35% the 15 and 80 mM KCl-induced contraction, respectively. Inhibition of mibefradil was consistent whatever the KCl concentration used (not shown). CH-IPA appeared thus hyperreactive due to T-VGCC stimulation by low KCl concentration, and this feature was reverted by specific T-VGCC inhibitors.

3.5 T-VGCC blockers reduce PASMC proliferation

Since cell proliferation is implicated in the development of PH,17 PASMC proliferation stimulated by 10% foetal calf serum was evaluated. In the presence of T-VGCC blockers, PASMC proliferation was inhibited in a concentration-dependent manner (Figure 6C). The anti-proliferative effect was still observed in the presence of the L-VGCC inhibitor, diltiazem. These data demonstrate the involvement of T-VGCC in PASMC proliferation.

4. Discussion

In this study, we have demonstrated that T-VGCCs play a role in the control of pulmonary vascular tone. We show, for the first time, that T-VGCC and mostly Ca\textsubscript{3.1} are involved in the development of PH since T-VGCC blockers administered in vivo or deletion of the Ca\textsubscript{3.1} gene prevent the development of CH-induced PH in rats and mice, respectively.

Patch-clamp experiments indicate that PASMCs express functional T-VGCC characterized by a rapidly inactivating current, a low-voltage activation threshold, and a sensitivity to T-VGCC inhibitors, such as mibefradil, TTA-A2, and NNC550396,9,24 as previously described in cerebral arteries.25 Initially described in few SMC types,17,21,24,26–28 the present study confirms that T-VGCCs are obviously present in more SMC types, for example PASMC.13 The molecular entity

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**Figure 3** Ca\textsubscript{3.1} channel is required for CH-induced PH in mice. WT and Ca\textsubscript{3.1}\textsuperscript{−/−} mice were maintained in the Nx condition or subjected to CH for 3 weeks (CH). (A) Mean RVP and (B) right ventricle hypertrophy (Fulton’s index). In (C), typical images of lungs from Nx WT, CH WT, Nx Ca\textsubscript{3.1}\textsuperscript{−/−}, and CH Ca\textsubscript{3.1}\textsuperscript{−/−} mice (the arrow indicates the vessel). (D) Quantification of wall thickness/vessels size ratio of pulmonary arteries. Note that Nx Ca\textsubscript{3.1}\textsuperscript{−/−} mice had the same parameters as Nx mice, but CH Ca\textsubscript{3.1}\textsuperscript{−/−} mice did not develop a severe PH, compared with WT CH mice. Asterisks indicate $P < 0.05$. ns: not significantly different.
responsible for the T-VGCC current in IPA is a combination of Cav3.1 and Cav3.2 isotypes (Figure 1D). Indeed, in the present patch-clamp experiments, low Ni$^{2+}$ concentrations (50 μM), which inhibit only Cav3.2 channel, partially inhibited the T-VGCC current. In some SMCs, the two isotypes display opposite effects on vasomotor response. Cav3.1 is mainly implicated in cell proliferation and contraction, whereas Cav3.2 is involved in vessel relaxation as in coronary arteries due to its action on potassium channels. If it was the case in PASMC, low Ni$^{2+}$ concentration, which inhibits Cav3.2, could unmask a vasoconstrictor effect mediated by Cav3.1 VGCC. Here, we did not observe such an effect, thus indicating that both Cav3.1 and Cav3.2 VGCCs are involved in IPA contraction. Patch-clamp experiments also show that TTA-A2 inhibits T-VGCC without affecting L-VGCC in PASMC, confirming that it is ~300-fold more selective for T-VGCC than for related L-VGCC. We therefore used this selective T-VGCC inhibitor for in vivo treatment of rats. TTA-A2 did prevent PH as it statistically reduced mean PAP increase, right cardiac hypertrophy, and pulmonary arterial remodelling that are hallmarks of PH. The different critical functions of T-VGCC including control of cell proliferation, vascular tone, and vascular hyperreactivity may explain the global effect of TTA-A2 on PH. T-VGCC inhibition reduces PASMC proliferation as in carotid or IPA, thus counteracting vascular wall remodelling.

We show that chronic treatment by TTA-A2 administered in vivo reduces pulmonary vascular hyperreactivity to both KCl and serotonin in CH-rats. This effect may be due to a continuous decrease in the calcium influx through T-VGCC, thus limiting vascular contraction and pressure increase. The subsequent lower calcium influx in IPA could also reduce calcium effect on IPA remodelling, which normally occurs during CH-PH. Indeed, T-VGCC expression is increased in CH-PASMC. Here, we did not observe a statistically significant increase in T-VGCC activity during PH in vitro in dissociated cell, but it could be hypothesized that such activity is increased, in vivo, by local modulators. For instance, serotonin, whose concentration is increased in PH, could activate T-VGCC via a fine tuning of the membrane potential. Additional signalling pathways could also account for the effect of T-VGCC inhibitors. For instance, hyperreactivity is linked to Rho-kinase-dependent pathway. Since interplays between T-VGCC and Rho-kinase have been previously described, T-VGCC inhibitors may also reduce hyperreactivity by interfering with this pathway. Modification of the expression of ionic channel (e.g. potassium channels), serotonin receptor, or intracellular calcium signalling pathway could not be excluded to explain the reduction in vascular hyperreactivity by TTA-A2. All T-VGCC inhibitors inhibited KCl-induced contraction to the same extent as or more weakly than that observed in Nx-IPA. The somewhat lower efficiency of blockers in CH-PASMC is consistent with the lower expression of T-VGCC in this model.
with the fact that membrane potential is more depolarized in CH-PASMC compared with Nx-PASMC, and this is associated with an extended inactivated state for T-VGCC (i.e. less available T-VGCC). TTA-A2 is a newly developed molecule and we have not examined its effect on all signalling pathways potentially involved in PH. The beneficial effect of TTA-A2 on PH development may also be due to an action on some other targets such as L-VGCC, Cl$^-$ channels, and TRP channels, which are involved in the pathophysiology of PH, although, in the latter case, we have shown that it did not inhibit TRPV4 channels (see Supplementary material online, Figure S2E).

We have confirmed the role of T-VGCC in PH using knock-out Cav3.1$^{-/-}$ mice. We have performed experiments on this strain mouse as (i) these mice have a normal blood pressure without important vessel abnormalities; (ii) relaxation phenomena are not altered; and (iii) SMC proliferation depends on this isotype. WT CH mice develop PH evidenced by an increase in systolic pressure, pulmonary arterial wall remodelling, and right ventricle hypertrophy (Figure 3). In contrast, mice lacking the Cav3.1 gene (Cav3.1$^{-/-}$) did not develop these cardiovascular alterations when exposed to CH. Cav3.1 deletion mimics TTA-A2 action in CH-rats and may act on PH by the same way. Other hypotheses may explain this prevention of PH. Deletion of T-VGCC in endothelium and cardiomyocytes may be involved in the reduction of PH symptoms. Indeed, T-VGCCs participate in the endothelium-dependent regulation of vascular tone and red blood cell aggregation and also in cardiac hypertrophy. Involvement of Cav3.2 channels in the development of PH could not be excluded and further experiments with Cav3.2$^{-/-}$ mice will be required to address this issue. Overexpression of Cav3.2 during PH is in favour of a putative role of this channel in PH. However, Cav3.2 VGCC may play a complex role since it is implicated in vascular relaxation in the coronary artery or it can antagonize relaxation elicited by 5,6-EETs (epoxyeicosatrienoic acids).

While the role of L-VGCC in IPA contraction has been extensively described the role of T-VGCC is not yet established. The function of T-VGCC was emphasized in our experiments by using a variety of T-VGCC inhibitors on KCl-induced contraction. KCl-induced contraction is of physiological relevance since it induces PASMC depolarization and VGCC activation is induced by several vasoconstrictor agents. In IPA, we show that TTA-A2 inhibits, in the concentration range specific to T-VGCC, KCl-induced contraction, confirming that T-VGCCs are important contributors to calcium influx in IPA. Previous studies have shown that TTA-A2 inhibits all three subtypes of low-voltage-gated T-VGCC with a comparable potency [half maximal inhibitory concentration (IC$_{50}$) $\sim$ 1 µM]. However, TTA-A2 is a more powerful inhibitor of Cav3.2 at more depolarized holding potential (IC$_{50}$ $\sim$ 10 nM). The
concentrations used in the present experiments are far greater and this does not allow to discriminate between the respective involvement of Cav3.1 and CaV3.2 isotypes in the contraction. NNC550396 is a less selective inhibitor of T-VGCC since it inhibits T- and L-VGCC as previously described. However, the observed inhibitory effect on NNC550396 at 1 μM was more important than that on L-VGCC, confirming the involvement of T-VGCC in contraction. Mibefradil inhibits T-VGCC current with a half maximal effective concentration (EC50) ≏ 1 μM on SMC but also other calcium channels according to the concentration used as shown in Figure 1. In PASMC, low concentrations of mibefradil (<1 μM) inhibited KCl-induced contraction to a larger extent than L-VGCC (Figures 1E and 3C), confirming the role of T-VGCC in KCl effect. Moreover, the additive effect of the combined application of diltiazem and NNC550396 (Figure 3C) confirms that the inhibition observed in the presence of less specific blockers of T-VGCC may be associated with a mixed inhibition of T-VGCC and L-VGCC current.

To further determine the role of T-VGCC in the contraction of IPA, we took into account the electrophysiological properties of these channels. Low depolarization activates mainly T-VGCC (low threshold channels), whereas high depolarization is required to activate L-VGCC as described in cerebral arteries. Taking advantage of the well-established relationship between extracellular KCl concentration and the resting membrane potential in IPA, we assumed that the contraction induced by low KCl concentrations (estimated depolarization of 8 and 16 mV for 15 and 30 mM KCl, respectively) implicates mainly T-VGCC, whereas high KCl concentrations (estimated depolarization of 28 and 38 mV for 40 and 80 mM KCl, respectively) activate mainly L-VGCC. In accordance with this hypothesis, TTA-A2 (and NNC550396) mainly inhibited the contraction induced by low KCl concentrations when compared with contraction induced by high KCl concentrations while less specific T-VGCC inhibitors, such as mibefradil, inhibited both contractions to the same extent. Activity of T-VGCC is dependent on the resting membrane potential value. Indeed, when the resting membrane potential is depolarized, T-VGCC turns into an inactivated state. Accordingly, T-VGCC blockers were less powerful when cells depolarize gradually (as during cumulative concentration response curve to KCl, Figure 5B). In conclusion, T-VGCC inhibition could thus reduce the calcium influx that triggers vasoreactivity and myogenic tone in the pulmonary artery as observed in the cerebral artery. Since the resting membrane potential of PASMC is around −50 mV, a value at which T-VGCCs are largely inactivated, the contribution of these channels to T-VGCC to the KCl-induced contraction might be questioned. However, a ‘window’ T-VGCC current as well as a T-VGCC-like current with a high threshold of activation might give rise to a sustained calcium influx also at more depolarized potentials. Both these currents are inhibited by TTA-A2.

Figure 6 T-VGCC blockers inhibit KCl-induced contraction in IPA from CH rat. (A) Effect of TTA-A2 at different concentration on non-cumulative concentration–response curve to KCl of CH pulmonary vessels. (B) Comparison of the percentage of inhibition of 30 mM KCl-induced contraction on Nx and CH pulmonary vessels by the T-VGCC blockers. Note that T-VGCC blockers have quite same efficiency on CH arteries compared with Nx arteries. (C) T-VGCC blocker reduces PASMC proliferation. Cell proliferation was assessed by the BrdU colorimetric method. PASMCs were incubated for 24 h in DMEM 10% foetal calf serum and blocker before BrdU incorporation. Data represent the mean ± SEM obtained in triplicate wells, performed in three rats. Asterisks indicate P < 0.05.
In conclusion, the present study indicates that T-VGCCs participate to IPA vascular reactivity under control conditions. Using different animal models of PH including genetically modified mice (Ca3.1−/− mice), we have also demonstrated that T-VGCCs, i.e. Ca3.1 channels, are implicated in the development of hypoxic PH. These results suggest that specific blockers of T-VGCC could be a valuable therapeutic means for PH.

**Supplementary material**

Supplementary material is available at Cardiovascular Research online.

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