Cellular disposition of transported polyamines in hypoxic rat lung and pulmonary arteries

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The polyamines putrescine, spermidine (SPD), and spermine are a family of low-molecular-weight organic cations essential for cell growth and differentiation and other aspects of signal transduction. Hypoxic pulmonary vascular remodeling is accompanied by depressed lung polyamine synthesis and markedly augmented polyamine uptake. Cell types in which hypoxia induces polyamine transport in intact lung have not been delineated. Accordingly, rat lung and rat main pulmonary arterial explants were incubated with [14C]SPD in either normoxic (21% O2) or hypoxic (2% O2) environments for 24 h. Autoradiographic evaluation confirmed previous studies showing that, in normoxia, alveolar epithelial cells are dominant sites of polyamine uptake. In contrast, hypoxia was accompanied by prominent localization of [14C]SPD in conduit, muscularized, and partially muscularized pulmonary arteries, which was not evident in normoxic lung tissue. Hypoxic main pulmonary arterial explants also exhibited substantial increases in [14C]SPD uptake relative to control explants, and autoradiography revealed that enhanced uptake was most evident in the medial layer. Main pulmonary arterial explants denuded of endothelium failed to increase polyamine transport in hypoxia. Conversely, medium conditioned by endothelial cells cultured in hypoxic, but not in normoxic, environments enabled hypoxic transport induction in denuded arterial explants. These findings in arterial explants were recapitulated in rat cultured main pulmonary artery cells, including the enhancing effect of a soluble endothelium-derived factor(s) on hypoxic induction of [14C]SPD uptake in smooth muscle cells. Viewed collectively, these results show in intact lung tissue that hypoxia enhances polyamine transport in pulmonary artery smooth muscle by a mechanism requiring elaboration of an unknown factor(s) from endothelial cells.

spermidine; endothelium; smooth muscle

The effects of chronic hypoxia on the lung are most prominent in the pulmonary arterial circulation, where there is extension of smooth muscle (or smooth muscle-like) cells into normally nonmuscular distal vessels, hypertrophy of smooth muscle cells in conduit pulmonary arteries (22–24), and alterations in the deposition of extracellular matrix proteins (7, 8, 21, 38). These anatomic changes, probably acting in concert with elevated vasomotor tone (27), lead to sustained increases in pulmonary vascular resistance and right ventricular hypertrophy.

The polyamines putrescine, spermidine, and spermine are a family of low-molecular-weight organic cations that contribute to many aspects of signal transduction. For example, although it has been recognized for many years that precise adjustments in cell polyamine pools are required for cell growth and differentiation (19), recent data indicate that the polyamines exhibit substantial antioxidant activity (9, 26), regulate certain ion channels and receptors (4, 5, 18), and seem to be required for a spectrum of mitochondrial functions (20, 30). Given the apparent central role of polyamines in regulating cell proliferative and functional states, their importance in governing responses of pulmonary vascular cells in hypoxia seems likely. Indeed, previous reports have shown that lung polyamine contents are elevated in hypoxia (28) and that pharmacological inhibition of this increase attenuates hypoxic pulmonary vascular remodeling and hypertension (1).

Mechanisms regulating pulmonary vascular cell polyamine contents in hypoxia are incompletely understood. In the hypoxic lung, de novo polyamine synthesis is depressed and polyamine uptake is augmented (10, 34). Thus the most likely basis for the increased lung polyamines required for hypoxia-induced changes in vascular structure is induction of transmembrane polyamine transport in effector cells of the pulmonary circulation. However, previous autoradiographic studies on the cellular localization of polyamine uptake in the normoxic lung (12–14, 31, 32, 35, 36) have uniformly noted that the most prominent sites of uptake are alveolar cells, especially type I and II pneumocytes. Uptake by cells of the pulmonary circulation has not been appreciated despite the fact that experiments in cultured human and bovine pulmonary artery endothelial (25, 37) and smooth muscle (2, 11) cells show that polyamine transport activity is indeed expressed in these populations.

The discrepancy between the lack of autoradiographic evidence for polyamine transport in the lung vasculature and observations in cultured endothelial and smooth muscle cells might be explained by at least...
two considerations. First, the findings in cultured pulmonary arterial cells could be an artifact of the experimental system and, by inference, increases in polyamine transport and content noted in the intact hypoxic lung occur in nonvascular cells. Second, constitutive polyamine transport by normoxic pulmonary arterial cells could be too low to be readily appreciated by autoradiography and must be augmented by hypoxia to be detected. Accordingly, the present experiments used lung and main pulmonary arterial explant preparations to test the hypothesis that hypoxia enhances polyamine uptake by cells of the pulmonary circulation. In addition, we used main pulmonary arterial explants and cultured rat pulmonary artery endothelial and smooth muscle cells to determine whether endothelial cells have an obligatory role in promoting polyamine uptake by hypoxic smooth muscle cells of the medial layer.

METHODS

Lung and main pulmonary arterial explant preparations. Male Sprague-Dawley rats, 210-250 g in weight, were anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg ip), and the heart and lungs were removed en bloc. An ~1-mm-thick section from the midplane of the lung was excised along with the main pulmonary artery. Both specimens were rinsed with cold PBS, cleaned of excess fatty tissue and blood, and immersed in complete culture medium containing DMEM, 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 0.1 mg/ml streptomycin. All culture medium constituents were obtained from GIBCO BRL (Life Technologies, Grand Island, NY). The explants were then placed in an incubator maintained at 37°C and gassed with 95% air and 5% CO₂ until hypoxic or normoxic exposure was initiated.

Some of the arterial segments were denuded of endothelium by gently abrading the intimal surface with a cotton swab. The absence of endothelium was verified by the lack of staining with an endothelial cell-specific lectin as described in Immunohistochemical and lectin histochemical detection of endothelial and smooth muscle cells. Hypoxic exposure. Denuded or intact artery segments and lung explants were assigned to normoxic or hypoxic incubator conditions. The hypoxic incubator (Forma Scientific, Marietta, OH) was maintained at 37°C and infused with 21% O₂, 5% CO₂, and 74% N₂, which resulted in PO₂ values ≥ 120 mmHg as measured by a Radiometer ABL30 blood gas analyzer (Copenhagen, Denmark). The hypoxic incubator was maintained under identical conditions except that the environment contained 2% O₂, resulting in a culture medium PO₂ of 27–35 mmHg.

Autoradiography. Rat main pulmonary arteries and peripheral lung explants were isolated, cultured for 24 h, and then exposed for an additional 24 h to normoxia or hypoxia as described in Hypoxic exposure. Subsequently, explants were removed from the incubation medium, rinsed with plain DMEM, and fresh medium containing DMEM along with 3 μM [³⁵S]spermidine trihydrochloride ([³⁵S]SPD; specific activity 116 mCi/mM; NEN) was added. The explants were then returned to the appropriate incubator for 6 h, after which they were removed, washed with PBS, and fixed in 10% Formalin for 24 h. Next, the explants were embedded in paraffin and sectioned serially in 5-µm slices. After removal of paraffin, the slides were dipped in warmed (45°C) Kodak NTB2 autoradiography emulsion, dried for 30 min, and stored for 2 wk at 4°C in the dark. The slides were then developed for 2 min in a 50% developer solution (Eastman Kodak, Rochester, NY), dried for 5 min, washed in tap water, and air-dried. Counterstaining was accomplished by a 30-s application of 1% toluidine blue solution and rinsing with tap water, followed by drying and mounting with coverslips. Slides were analyzed by light microscopy for silver grain detection.

[³⁵S]SPD uptake in pulmonary arterial explants. Arterial rings were isolated as described in Lung and main pulmonary arterial explant preparations and placed in normoxic or hypoxic incubators for up to 48 h. For [³⁵S]SPD uptake studies, rings were removed from medium and immersed in plain DMEM to avoid interference by serum polyamines. The specific uptake of [³⁵S]SPD in intact tissue was determined as described previously (34). In brief, pairs of pulmonary arterial rings were incubated at 37°C under normoxic or hypoxic conditions with either 3 μM [³⁵S]SPD or 2.5 mM [³H]sucrose for the indicated durations. Total uptakes of [³⁵S]SPD and [³H]sucrose were normalized to tissue wet weight, and the tissue space [³H]sucrose was subtracted from that for [³⁵S]SPD to calculate specific [³⁵S]SPD uptake.

Immunohistochemical and lectin histochemical detection of endothelial and smooth muscle cells. Deparaffinized slides were incubated for 20 min in PBS with 0.5% BSA, 0.05% NaN₃, and 0.005% Tween, pH 7.2 (PBS solution), with 1% fetal bovine serum and 0.05% H₂O₂ (to block nonspecific reactivity and extinguish endogenous peroxidases). Slides were washed for 5 min in PBS solution and incubated for 1 h with monodonal anti-actin antibody (Sigma) or polyclonal anti-factor VIII-related antigen (FVIII-RAG) antibodies (DAKO, Carpinteria, CA) diluted 1:200 in PBS solution. Immunoreactivity was developed with a LABS 2 kit (DAKO) following the manufacturer’s protocol. Because FVIII-RAG may diffuse into the intima, an additional staining with sialic acid-specific lectin from Tritrichomonas mobilensis (TML), which detects luminal membrane sialoglycoproteins, was used to detect endothelial cells (3). Deparaffinized slides were humidified in PBS solution for 5 min followed by incubation with 10 μg/ml of biotinylated TML (Calbiochem-Novabiochem, San Diego, CA) for 1 h. After two 5-min washes in PBS solution, the slides were incubated with streptavidin-peroxidase complex (DAKO) and color product was developed with 3-amino-9-ethylcarbazole (DAKO). All slides were counterstained with hematoxylin. Slides without application of primary antibodies served as negative controls.

Rat main pulmonary artery endothelial and smooth muscle cell cultures. Main pulmonary arteries were isolated from 300-g Sprague-Dawley rats killed with an overdose of Nembutal. Isolated arteries were opened, and the intimal lining was carefully scraped with a scalpel. The harvested cells were then placed into T25 Corning flasks in F-12 nutrient mixture and DMEM (1:1) supplemented with 10% FBS, 100 U/ml penicillin, and 0.1 mg/ml streptomycin (GIBCO BRL). Culture medium was changed once a week, and, after reaching confluence, the cells were harvested with a 0.05% solution of trypsin (GIBCO BRL) and passed up to 15 times. The endothelial cell phenotype was confirmed by acetylated low-density lipoprotein (LDL) uptake, factor VIII immunocytochemical staining, and the lack of immunostaining with smooth muscle cell α-actin antibodies (Sigma).

Denuded main pulmonary artery specimens were placed in the same medium described above for isolation and culture of endothelial cells. After 3 days under standard incubator conditions, the arterial specimens were removed, and cells that had migrated from the explants were grown to confluence. Culture medium was changed every 3 days. Cells were harvested with a 0.05% solution of trypsin, and passages
Hypoxic exposure and assessment of \(^{[14C]}\)SPD in cultured main pulmonary artery endothelial and smooth muscle cells. Endothelial and smooth muscle cells (1 × 10^6 cells in 2 ml medium/well) were seeded in 12-well plates (Costar, Cambridge, MA). Polymamine transport experiments were performed after 2–3 days, when the cells reached 95–100% confluence. The plates were placed in incubators for 24 h (Forma Scientific) and purged with gas mixtures containing either 21 or 2% \(O_2\), 5% \(CO_2\), and balance \(N_2\). Medium \(Po_2\) values, determined with a Radiometer model ABL30 blood gas analyzer, were ≈120 mmHg for the control cultures and 27–35 mmHg for cultures designated as hypoxic.

After incubation under the indicated conditions, cells were rinsed with serum-free DMEM, after which 1 ml of DMEM was added to each well and the cells were allowed to acclimate for 30 min. Subsequently, 3 \(\mu\)M \(^{[14C]}\)SPD was added to each well, and the cells were then incubated for 30 min, a duration that preliminary experiments indicated was in the linear range of uptake. Nonspecific SPD transport was estimated as the uptake occurring in cells incubated at 4°C. At the appropriate time, medium containing residual \(^{[14C]}\)SPD was aspirated, and cells were rinsed twice with cold PBS and overlaid with 1.5 ml of PBS solution containing 0.5% SDS for 30 min. The cell lysates were then transferred to scintillation vials and mixed with 4 ml of scintillation cocktail (Beckman Instruments, Fullerton, CA), and radioactivity was determined with a Beckman LS 6500 liquid scintillation counter. The \(^{[14C]}\)SPD uptake rates were calculated in terms of nanomoles per 1 million cells per minute.

Statistical analysis. Quantitative data are presented as means ± SD. One- or two-way ANOVAs, depending on the experimental designs, combined with Newman-Keuls tests, were used to detect significant differences between experimental groups. \(P\) values < 0.05 were considered to denote significance.

RESULTS

As reported by others (12–14, 31, 32, 35, 36), we found that a prominent site of \(^{[14C]}\)SPD uptake in both normoxic and hypoxic lung explants was the alveolar wall, with a punctate labeling pattern in corners of alveoli suggestive of type II pneumocyte involvement (data not shown). We did not pursue this observation insofar as no dramatic change in the extent of labeling was noted on hypoxic exposure.

Figure 1 shows autoradiographs of \(^{[14C]}\)SPD uptake in normoxic and hypoxic lung explants, with particular attention devoted to localization in conduit (lobar) and fully and partially muscularized pulmonary arteries. Labeling of vascular structures was subtle in normoxic lung explants, but hypoxia caused a prominent increase in \(^{[14C]}\)SPD uptake in all three vessel types in hypoxia. The increased labeling density was most conspicuous in the medium, with less impressive increases in uptake associated with the intimal or adventitial layers.

We next determined whether pulmonary arterial explants exhibited time-dependent increases in \(^{[14C]}\)SPD uptake when cultured under hypoxic conditions. As shown in Fig. 2, both normoxic and hypoxic pulmonary arterial explants accumulated \(^{[14C]}\)SPD, with hypoxic preparations sequestering more \(^{[14C]}\)SPD at all but the 1-h duration of hypoxic exposure. Autoradiography was used to determine cell types within the arterial explants that sequestered \(^{[14C]}\)SPD after 24 h of exposure to normoxic or hypoxic culture environments. The representative photomicrographs shown in Fig. 3, top, show that there were modest degrees of intimal and adventitial labeling in normoxic vessels but that the most impressive localization was in the medial arterial layer. More importantly, although neither intimal nor adventitial labeling was dramatically increased with hypoxic exposure, there was a prominent elevation in medial \(^{[14C]}\)SPD uptake in hypoxic arterial explants.

Two complementary experiments were performed to determine involvement of endothelial cells in the increased medial arterial uptake of \(^{[14C]}\)SPD noted in hypoxic pulmonary explants. First, the effect of intimal denudation on hypoxia-induced increases in \(^{[14C]}\)SPD uptake was examined using both tissue radioactivity determination and autoradiography as outcome measures. As shown in Fig. 4, endothelial denudation, confirmed by the absence of endothelial cell-specific lectin immunostaining (Fig. 3, bottom), was accompanied by an ~50% reduction in \(^{[14C]}\)SPD uptake in the normoxic state and by the failure of hypoxia to increase uptake as it does in the presence of the intimal layer. The autoradiographs shown in Fig. 3, middle, indicate that denuded normoxic explants exhibit less impressive medial localization of \(^{[14C]}\)SPD than vessel explants with a normal intimal layer and that in the absence of endothelium, hypoxia fails to increase the density of uptake in the arterial medial layer.

To determine whether an endothelial cell-derived factor(s) was required for the hypoxic induction of polyamine transport by pulmonary arterial smooth muscle cells, medium conditioned by endothelial cells cultured in normoxia or hypoxia for 24 h was applied to denuded pulmonary arterial explants. Polyamine uptake in normoxic or hypoxic explants was assessed 24 h after application of the endothelial cell-conditioned medium. As shown in Fig. 5, neither normoxic nor hypoxic endothelial cell-conditioned medium restored \(^{[14C]}\)SPD uptake in normoxic denuded explants to the same level as in intact pulmonary arteries. It is important, however, that although medium conditioned by normoxic endothelial cells failed to engender \(^{[14C]}\)SPD uptake by hypoxic denuded explants, addition of hypoxic endothelial cell-conditioned medium was associated with a substantial increase in uptake when denuded explants were cultured in hypoxic conditions.

A final series of experiments was conducted to determine whether cultured rat main pulmonary artery endothelial and smooth muscle cells would recapitulate actions of hypoxia observed in the pulmonary arterial explant preparation. Endothelial and smooth muscle cells were cultured in normoxia and hypoxia for 24 h, after which the uptake of 3 \(\mu\)M \(^{[14C]}\)SPD was determined. As shown in Fig. 6, although hypoxic endothel-
lial cells exhibited a slight, but significant, increase in the uptake rate of \([^{14}C]\)SPD relative to normoxic cells, there was no effect of hypoxia in cultured smooth muscle cells. We next performed a crossover experiment in which \([^{14}C]\)SPD uptake rate was assessed in normoxic and hypoxic smooth muscle cell cultures exposed for 24 h to medium conditioned by either normoxic or hypoxic endothelial cells. The results, also depicted in Fig. 6, indicate that addition of normoxic or hypoxic endothelial cell-conditioned medium failed to alter \([^{14}C]\)SPD uptake rate in normoxic smooth muscle cells. However, application of normoxic endothelial cell-conditioned medium was associated with a modest hypoxia-induced increase in \([^{14}C]\)SPD transport in smooth muscle cells, whereas medium conditioned by hypoxic endothelial cells tended to promote a larger increase in \([^{14}C]\)SPD uptake.

**DISCUSSION**

Previous reports (12–14, 31, 32, 35, 36) have established that cells of the alveolar wall avidly sequester polyamines from the extracellular environment. Polyamine transport by pulmonary vascular cells in the intact lung has not been appreciated. Nevertheless, pulmonary vascular cells in culture do indeed exhibit polyamine transport systems (2, 11, 25, 37) that are upregulated by important pathological stimuli includ-
ing hypoxia (2, 11). Hypoxia also elevates polyamine uptake by intact lungs (34), but the cell types in which this response occurs have not been delineated. These considerations underscore the central objective of this study, namely, to determine whether hypoxia increases polyamine uptake by pulmonary vascular cells in an environment more closely approximating the intact lung.

Our initial experiments employed rat lung explant preparations similar to those used in earlier studies (12–14) to define the cellular basis of lung polyamine uptake. Indeed, although not a focus of the present work, we noted considerable [14C]SPD localization in alveolar wall cells, probably type I and type II pneumocytes, in both normoxic and hypoxic lungs. Localization in vascular structures was modest in comparison. However, when lung explants were cultured in a hypoxic environment for 24 h, there was a prominent increase in the density of [14C]SPD in the medial layers of conduit, muscularized, and partially muscularized pulmonary arteries. Because hypoxia causes remodeling of each of these vascular segments (22–24), it is likely that induction of polyamine transport plays a critical role in governing changes in cell polyamine content required for the specific adaptive response.

To provide further evidence that hypoxia acts directly on the pulmonary arterial wall to enhance polyamine uptake by resident cells, we quantified the extent of [14C]SPD uptake in pulmonary arterial explant preparations and used autoradiography to discern the cellular basis of uptake. Similar to findings in intact lung tissue, we found that arterial explants sequestered [14C]SPD in a time-dependent manner and that hypoxia markedly augmented the extent of uptake. In addition, although both intimal and medial arterial layers were labeled by [14C]SPD, the hypoxia-induced increase in transport was most evident in smooth muscle cells of the media. Viewed collectively, these findings in both lung and pulmonary arterial explant preparations indicate that hypoxia increases polyamine uptake in pulmonary arterial cells, most conspicuously in vascular smooth muscle.

The stimulus for hypoxia-induced increases in vascular [14C]SPD uptake is unclear. Karl et al. (17) and Rannels (29) showed that, after pneumonectomy, the increased polyamine uptake in the remaining lung is probably driven by alterations in mechanical forces. Altered mechanical forces acting on vessel walls as a consequence of hypoxic pulmonary vasoconstriction in the blood-perfused pulmonary circulation clearly cannot be a factor in the explant preparations used in the present studies. Instead, it appears that hypoxia either directly acts on lung vascular cells to enhance polyamine transport or alters the cellular environment such that the uptake system is activated.

We detected [14C]SPD localization in both intimal and medial arterial cells, but the increase in labeling caused by hypoxia was most evident in the medial layer. To explore specific involvement of endothelial cells, we examined the impact of hypoxia in denuded pulmonary arterial explants. Not only was the baseline uptake reduced by endothelial denudation, but the increase normally evoked by hypoxia was abolished. This observation suggests that endothelial cells are either major sites of [14C]SPD uptake in both normoxia and hypoxia or that these cells elaborate a factor(s) that increases [14C]SPD uptake by the underlying smooth muscle. Keeping in mind potential spurious effects of the denudation procedure per se on polyamine transport, we believe that the prominent medial uptake of [14C]SPD in normoxic and hypoxic explants combined with inhibition of hypoxia-induced increases in medial [14C]SPD uptake by endothelial denudation supports the latter possibility.

To determine potential involvement of soluble factor(s) released by endothelial cells that could impact on [14C]SPD uptake by medial arterial smooth muscle, we applied endothelial cell-conditioned medium to denuded pulmonary arterial explants. A crossover design was used in which medium conditioned by normoxic or hypoxic endothelial cells was applied to either normoxic or hypoxic arterial explants. Normoxic endothelial cell-conditioned medium failed to increase the [14C]SPD uptake rate in either normoxic or hypoxic denuded explants. Thus it seems unlikely that hypoxic induction of polyamine transport in smooth muscle cells can be ascribed to activation of a factor(s) released constitutively by cultured normoxic endothelial cells. On the other hand, although medium conditioned by hypoxic endothelial cells also did not elevate the uptake rate in normoxic explants, it permitted substantial increases in hypoxic preparations. This pattern of results supports the idea that a factor(s) elaborated by hypoxic endothelial cells is permissive for the ability of hypoxia to increase the [14C]SPD uptake rate in pulmonary arterial smooth muscle.

Our findings in main pulmonary arterial explant preparations were recapitulated in cultured endothelial and smooth muscle cells. Endothelial cell [14C]SPD uptake was slightly, but significantly, enhanced by culture in a hypoxic environment, whereas main pulmonary artery smooth muscle [14C]SPD uptake was unaffected by hypoxia unless endothelial cell-conditioned
medium was added. Medium conditioned by normoxic endothelial cells was accompanied by a slight but significant induction of hypoxic smooth muscle cell [14C]SPD uptake, whereas medium conditioned by hypoxic endothelial cells was linked to a substantially larger hypoxia-induced increase in transport. These observations also support the notion that hypoxic endothelial cells release a factor(s) that engenders polyamine transport induction by hypoxia in pulmonary artery smooth muscle cells.

The results from this study raise several questions about mechanisms of lung vascular polyamine transport regulation in hypoxia. First, and perhaps most interesting, the identity of the postulated endothelial cell-derived factor(s) that engenders hypoxic polyamine uptake by underlying smooth muscle is unknown. There are many candidate mediators, including, to name a few, endothelin, platelet-derived growth factor, matrix metalloproteinases, and numerous vasoactive factors. Our current data point to at least one apparent requirement for filling this suspected permissive role, namely, that the endothelium-derived factor(s) by itself does not substantially elevate smooth muscle polyamine transport, but rather, it enables the ability of hypoxia to do so. This paradigm is consistent with a report by Dempsey et al. (6), who showed that neither protein kinase C activation with phorbol myristate acetate nor hypoxia by themselves caused impressive
proliferation in cultured pulmonary artery smooth muscle cells but that protein kinase C activation primes the cells such that they exhibit a brisk proliferative response to subsequent hypoxic exposure.

The present study also points to an interesting discrepancy between polyamine transport regulation in cultured bovine and rat pulmonary artery smooth muscle cells. Previous work by Aziz et al. (2) and Haven et al. (11) with bovine cells showed that hypoxia was capable of promoting large increases in transport of all three polyamines without the requirement of adding endothelial cell-conditioned medium to the smooth muscle culture system. On the other hand, in the present study, we found that rat pulmonary artery smooth muscle cells failed to respond to hypoxia with an increase in [14C]SPD uptake unless medium conditioned by endothelial cells was added. One explanation for this difference is that rat and bovine pulmonary artery smooth muscle cells differ with respect to polyamine transport regulation. Although this has not been tested directly, it has previously been noted (33) that there are important species- and cell type-dependent differences in polyamine regulatory pathways. It is also possible that bovine smooth muscle cells in culture are phenotypically modulated such that they respond in a manner similar to the intact vessel but do so independent of endothelial cell-derived factors. For example, the matrix on which the smooth muscle cells reside is an important determinant of functional state (15, 16), and the matrix environment of cultured cells is clearly different from the normal vessel wall. Additional studies will be required to determine the mechanisms whereby pulmonary artery smooth muscle cells from the rat and cow differ in terms of hypoxic induction of polyamine transport.

In bovine pulmonary artery smooth muscle cells, hypoxia upregulates at least two discrete polyamine transporters: one for spermine and spermidine and another for all three polyamines. The elevation is driven by a two- to threefold increase in the maximal uptake while Michaelis-Menten constant values are increased slightly (2). This study is one of the first to explore polyamine transport in rat endothelial and smooth muscle cells, and we now know only that hypoxia increases the specific uptake of a single polyamine (SPD) at a single concentration. Questions arising from this observation include whether there are multiple polyamine transporters in rat pulmonary artery smooth muscle cells as there are in bovine cells and whether hypoxia increases transport through an effect on the transporter system maximal uptake.

In summary, this study shows in intact lung tissue and pulmonary arteries that hypoxia augments polyamine transport in cells of the pulmonary circulation, probably vascular smooth muscle. This adds to the lung cell populations known to express polyamine transport pathways that are regulated by physiological and pathological stimuli. In addition, our data suggest that endothelial cell-derived factors play a permissive role in governing the polyamine transport response to hypoxia in the underlying smooth muscle. The precise mechanism whereby endothelial and smooth muscle cells interact in hypoxia to regulate this important signaling pathway remains to be determined, but the fact that rat cultured main pulmonary artery endothelial and smooth muscle cells recapitulate the behavior of the intact pulmonary artery suggests that these in vitro systems could prove useful in exploring the possibilities.
REFERENCES


