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ROLE OF ATP AND SODIUM IN POLYAMINE TRANSPORT IN BOVINE PULMONARY ARTERY SMOOTH CELLS

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Abstract—Increased polyamine transport may be a key mechanism driving elevations in lung cell polyamine content necessary for the development of chronic hypoxic pulmonary hypertension. Bovine pulmonary artery smooth muscle cells (PASMCs) in culture exhibit two carriers for polyamines, a non-selective one shared by the three polyamines, putrescine (PUT), spermidine (SPD), and spermine (SPM), and another that is selective for SPD and SPM. Hypoxia appears to up-regulate both carriers. In this study, we examined the role of ATP and the Na^+ gradient in regulating polyamine transport in control PASMCs and in PASMCs with polyamine transport augmented by culture under hypoxic conditions (Po_2: 15–30 torr). Inhibition of ATP synthesis with dinitrophenol + iodoacetate profoundly reduced polyamine uptake in both control and hypoxic PASMCs. Putrescine uptake was somewhat more sensitive to iso-osmotic replacement of extracellular Na^+ with choline chloride or sucrose than were SPD or SPM in both hypoxic and standard cells, but under no conditions did Na^+ replacement substantially alter polyamine uptake. Treatment of PASMCs with ouabain, a Na^+-K^+ATPase inhibitor, or with gramicidin, a Na^+ ionophore, minimally attenuated polyamine transport, whereas the Na^+/K^+ ionophore monensin increased polyamine uptake in standard, but not in hypoxic, cells. In general, the reduction in the extracellular Na^+ content or ionophore-induced increases in Na^+ permeability had a greater suppressive effect on polyamine transport in hypoxic cells than in standard cells, suggestive of the induction of Na^+-dependent polyamine carriers by hypoxia. These observations indicate that the activities of the two putative polyamine transport pathways in standard PASMCs, as well as their up-regulation by hypoxia, require ATP synthesis. In addition, it appears that polyamine transport in PASMCs is composed of two components: one a prominent sodium-independent transporter and the other a relatively minor component that is sodium dependent. The latter may be activated by hypoxic exposure in combination with the induction of new polyamine carriers.

Key words: pulmonary hypertension; hypoxia; signal transduction

The polyamines, PUT†, SPM and SPD, are a family of low molecular weight organic cations that are necessary for cell growth and differentiation [1]. Two general mechanisms seem to regulate intracellular polyamine content. *De novo* polyamine biosynthesis involves the coordinated interaction of several enzymes with the initial enzyme in the cascade, ODC, serving as one of the key, rate-limiting steps. Along with *de novo* polyamine synthesis, many cell types also exhibit a carrier-mediated transporter(s) for the polyamines [2]. There seems to be considerable heterogeneity in the nature of these transport pathways; some cells express multiple transporters with overlapping selectivities, while others exhibit a single non-selective pathway. The energetics of polyamine transport also may be cell type- or stimulus-specific, with both ATP and the sodium gradient required for import of polyamines.

Increases in cellular polyamine levels are associated with a variety of physiologic and pathologic events in the lung. Elevation of lung cell polyamine contents required for postnatal lung development [3], compensatory lung growth after pneumonectomy [4], repair after hyperoxic lung injury [5], and monocrotaline-induced lung injury and pulmonary hypertension [6, 7] are accompanied by increases in ODC activity and thus seem to be driven, in part, by induction of *de novo* polyamine synthesis. In contrast, chronic hypoxic pulmonary hypertension is associated with down-regulation of *de novo* polyamine synthesis and an increase in transmembrane polyamine transport [8]. Cultured PASMCs exhibit a similar response to hypoxic exposure; ODC activity and mRNA levels are depressed, while SPD uptake is elevated [9]. Based on competition experiments, we believe that there are two polyamine uptake pathways in PASMCs, one selective for SPD and SPM, and another one utilized by all three polyamines. Both transporters in PASMCs are induced by hypoxia [10].

Multiple lines of evidence suggest that ATP synthesis is required for expression of polyamine

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† Abbreviations: PUT, putrescine; SPM, spermine; SPD, spermidine; ODC, ornithine decarboxylase; PASMCs, pulmonary artery smooth muscle cells; DMEM, Dulbecco's Modified Eagle's Medium; DNP, dinitrophenol; IA, iodoacetate.

transport activity [2]. The role of the Na^+ gradient as an energy source in polyamine transport is unclear, partly because the involvement of Na^+ may be cell type- and stimulus-specific. For example, it has been demonstrated in type II pneumocytes that uptake of PUT and SPD, but not SPM, was sensitive to extracellular Na^+ concentration [11]. In cultured human umbilical vein endothelial cells, PUT and SPD uptake was found to be partially dependent on Na^+, whereas SPM uptake was Na^+ independent [12]. On other hand, persuasive evidence indicates that polyamine uptake is not driven by the Na^+ electrochemical gradient in adrenocortical cells [13], B16 melanoma cells [14] and isolated rat hepatocytes [15]. The failure to identify a Na^+-dependent component of uptake in these latter cell types may be related to the fact that induction of this pathway for polyamine transport may be stimulus-specific. In support of this possibility, renal LLC-PK_1 cells express both sodium-dependent and -independent polyamine uptake, with the former disproportionately stimulated by epidermal growth factor in combination with insulin [16].

In light of the above considerations, the present study determined whether both ATP and Na^+ play a role in the regulation of the two putative polyamine carriers in PASMCs. In addition, we sought to determine if hypoxia, a pathologically relevant stimulus for polyamine uptake in the intact lung and in PASMCs, utilizes a specific Na^+-dependent polyamine uptake pathway that differs from those transporters operative in the standard state. To address these issues, we examined the impact of Na^+ substitution, inhibitors of the Na^+-K^+ ATPase and two different Na^+ ionophores on polyamine uptake rate in cells cultured under standard conditions and in hypoxic PASMCs.

MATERIALS AND METHODS

Materials. DMEM, trypsin-EDTA, and penicillin/streptomycin solution were purchased from Gibco (Grand Island, NY). Defined bovine calf serum was provided by HyCLone (Logan, UT). Phosphate-buffered saline and other drugs and chemicals used in this study were purchased from the Sigma Chemical Co. (St Louis, MO). Radiochemicals and related supplies used in assessment of polyamine transport were purchased from the Amersham Corp. (Arlington Heights, IL). All plasticware used for tissue culture was obtained from Costar (Cambridge, MA).

Pulmonary artery smooth muscle cell cultures. Bovine main pulmonary arteries were harvested from freshly slaughtered cattle (Dawson's Packing Co., Louisville, KY), immersed in cold (4°) Pucks F6 solution supplemented with 300 U/mL penicillin, 0.3 mg/mL streptomycin, and transported to the laboratory. The arteries were then opened longitudinally, and the endothelium was scraped away. Medial explants, approximately 2 mm × 2 mm, were dissected from the subintima and plated in culture flasks containing DMEM supplemented with 10% defined fetal bovine serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. Cells were then grown to confluence and propagated in culture. Cells from eight different adult cows were used in these experiments. Culture medium was changed every 3–4 days. Cells were harvested using a 0.05% solution of trypsin. All experiments utilized cells from passages between 2 and 10. The smooth muscle phenotype of the cells was confirmed by the presence of smooth muscle-specific actin, as evidenced by immunocytochemical analysis (data not shown). Cell counts were determined by hemocytometry. Cell viability was assessed using trypan blue exclusion according to standard techniques.

Hypoxic exposure. To examine the effects of hypoxia on polyamine transport and ODC activity, PASMCs were seeded into 35 mm^2, 6-well cluster plates (Costar) at a density of 75,000/well and cultured in DMEM supplemented with 10% serum for 24 hr. Subsequently, the well plates were placed in specially constructed plastic chambers affixed with inflow and outflow ports that permitted the chambers to be exposed to selected gas mixtures. The chambers were then placed in standard tissue culture incubators after which they were exposed for an additional 24 hr to one of two levels of environmental oxygen: "standard" conditions, wherein the cells were maintained in incubator chambers gassed with 95% air–5%CO_2 ($Po_2 > 100$ torr), and "hypoxic" conditions, where the incubators were purged with 0% O_2, 5% CO_2, and 95% N_2 ($Po_2 = 15$–30 torr). Aliquots of culture medium (1.0 mL) were withdrawn anaerobically at termination of hypoxic exposure, and Po_2 values were determined using an Instrument Laboratories model 213 blood gas analyzer (Oxnard, CA).

Polyamine transport. After the 24-hr exposure to hypoxia or standard tissue culture conditions, PASMCs were rinsed with fresh, serum-free DMEM after which 1 mL of serum-free DMEM was added to each well and the cells were allowed to acclimate for a 1-hr period. A selected [^{14}C]-polyamine was added to each well in a concentration of 3 μM, and cells were incubated for 15 min and at temperatures of 4° and 37°. Medium containing residual [^{14}C]-polyamine was aspirated, and cells were placed on ice and rinsed with cold phosphate-buffered saline. In experiments utilizing the metabolic inhibitors DNP (0.1 mM) and IA (1.0 mM), the compounds were added 15 min before the addition of a radioactive polyamine. PASMCs were then digested for 1 hr at room temperature in 1 N NaOH. Prior to determination of cell-associated radioactivity, 400 μL of the cell digest was neutralized with 400 μL of 1 N acetic acid. An additional 400 μL of distilled H_2O along with 2 mL of scintillation fluid was added to the neutralized digests, and radioactivity was determined using a Packard liquid scintillation counter (Downers Grove, IL). We elected to normalize [^{14}C]-polyamine content and uptake rate in terms of cell number rather than protein or DNA content since culture under hypoxic conditions may alter these biochemical parameters [15, 16].

Effect of extracellular Na^+ *replacement.* The control medium used in these experiments has been described previously [10] and had the following composition: 116 mM NaCl, 1 mM NaH_2PO_4, 2 mM Na_2HPO_4, 5.4 mM KCl, 1 mM $CaCl_2$, 0.8 mM $MgCl_2$, 5.5 mM glucose, and 20 mM HEPES, pH 7.4.

Two other media were prepared in which NaCl was replaced iso-osmotically with either choline chloride (ChCl) or sucrose. KH_2PO_4 and K_2HPO_4 were substituted for the corresponding Na^+-phosphate salts. Media with Na^+ contents ranging from 0 to 100% of control were prepared by mixing NaCl buffer with one of the other two buffers. PASMCs were incubated in media with reduced Na^+ content for 1 hr prior to assessment of polyamine uptake. The effects of Na^+ replacement were examined in cells cultured at both 37° and 4° so that the effect of Na^+ substitution on non-specific uptake or adsorption of the polyamines, as detected at the lower temperature, could be excluded from the calculation of uptake rates. The specific component of polyamine uptake was calculated by subtracting the polyamine uptake rate values at 4° from those generated at 37° at each polyamine concentration.

Pharmacologic modulation of the sodium gradient. The following pharmacologic agents were used to modulate the sodium gradient: ouabain (0.01–1.0 mM), a Na^+-K^+ ATPase inhibitor; gramicidin (10–200 nM), a Na^+ ionophore; and, monensin (1–2 μM), a Na^+/K^+ ionophore. All agents were added 15 min prior to assessment of polyamine uptake. Similar to the Na^+ replacement study, the effects of these drugs were assessed on cells cultured at both 37° and 4°, and the specific polyamine uptake was calculated as noted previously.

Statistical analyses. All values are presented as means ± SEM. Data were compared between experimental groups using one- or two-way analysis of variance combined with the Neuman–Kuels test, depending on the specific experiment. In all cases, P values <0.05 were considered to denote statistical significance.

RESULTS

ATP-dependent polyamine uptake. To determine whether polyamine transport was ATP-dependent, PASMCs cultured under standard and hypoxic conditions were pretreated with a combination of 0.1 mM DNP and 1 mM IA to inhibit ATP synthesis secondary to oxidative phosphorylation and glycolysis. The brief 15-min exposure to the metabolic inhibitors was not associated with appreciable cell death as evidenced by no differences in trypan blue exclusion (data not shown). The effect of inhibition of ATP synthesis on polyamine transport rate in cells cultured under standard and hypoxic conditions is shown in Fig. 1. Consistent with our previous reports [9, 10], hypoxia increased uptake of all three polyamines in control cells. Pretreatment with DNP + IA blocks the specific component of polyamine uptake in both standard and hypoxic cells [10]. Metabolic inhibition reduced PUT transport in PASMCs cultured under standard conditions to levels observed in cells exposed to 4° (data not shown), and abolished the increase in uptake evoked by hypoxia. SPD uptake in cells cultured under standard conditions also was reduced to levels approximating those at 4° by the metabolic inhibitors. SPD uptake in hypoxic PASMCs was suppressed by metabolic inhibition, but not to the same extent observed in cells cultured under standard conditions. As it has been reported previously [10], cultured PASMCs exhibited a substantial degree of non-specific SPM uptake over the specific uptake component, as evidenced by the absence of saturation kinetics as the concentration of SPM was increased and by the fact that metabolic inhibition, as well as low temperature [10], failed to suppress uptake markedly. Total SPM uptake was increased slightly by culture under hypoxic conditions, and this hypoxic-induced increase was depressed by DNP/IA.

Effect of extracellular sodium on polyamine transport. To examine the effect of extracellular Na^+ on the specific component of polyamine uptake, NaCl was iso-osmotically replaced with either choline chloride or sucrose, and the specific uptake rate of 3 μM of each polyamine was determined in cells cultured under standard conditions or with polyamine transport augmented by exposure to hypoxia. Neither mode of Na^+ replacement substantially altered polyamine uptake in PASMCs, including the non-specific component of uptake assessed at 4° (data not shown). As shown in Fig. 2, substitution of 25% of the normal culture medium Na^+ content with choline chloride suppressed PUT uptake by approximately 25%, whereas greater degrees of Na^+ substitution were required to produce a similar reduction in specific SPD and SPM transport. In no instance was transport reduced beyond 40%, even when Na^+ was completely excluded from the culture media. The effect of replacing Na^+ with choline also was not concentration-related. There were no major differences between control and hypoxic cells in terms of the modest impact of choline substitution for Na^+. Figure 3 shows the effect of NaCl replacement with sucrose on polyamine transport. PUT uptake was again more sensitive to Na^+ replacement than SPD and SPM transport. In addition, Na^+ substitution had a greater influence on polyamine transport in hypoxic cells versus control cells. PUT uptake was reduced in hypoxic PASMCs at 25% replacement of Na^+, while uptake in standard cells was reduced significantly at 50% Na^+ replacement. SPD uptake was reduced exclusively in hypoxic cells but only when 75% or more of the Na^+ content was replaced by sucrose. SPM uptake in hypoxic cells was unaffected by Na^+ substitution, but uptake in standard cells was increased by 35–60%. While both choline and sucrose substitutions for Na^+ were similar in terms of their modest inhibition of PUT uptake, they differed in two key respects: while sucrose substitution inhibited SPD uptake in hypoxic cells (but not standard cells), choline substitution was without effect at either environmental oxygen content. Additionally, while choline substitution had a slight inhibitory effect on SPM uptake, particularly in hypoxic cells, sucrose substitution failed to inhibit uptake and instead increased SPM transport in standard cells. It is important to note that neither choline nor sucrose, even when completely replacing the normal Na^+ content, reduced transport of any of the polyamines by more than 50%, regardless of whether the cells were cultured under standard or hypoxic conditions.

Effect of pharmacologic manipulation of the Na^+

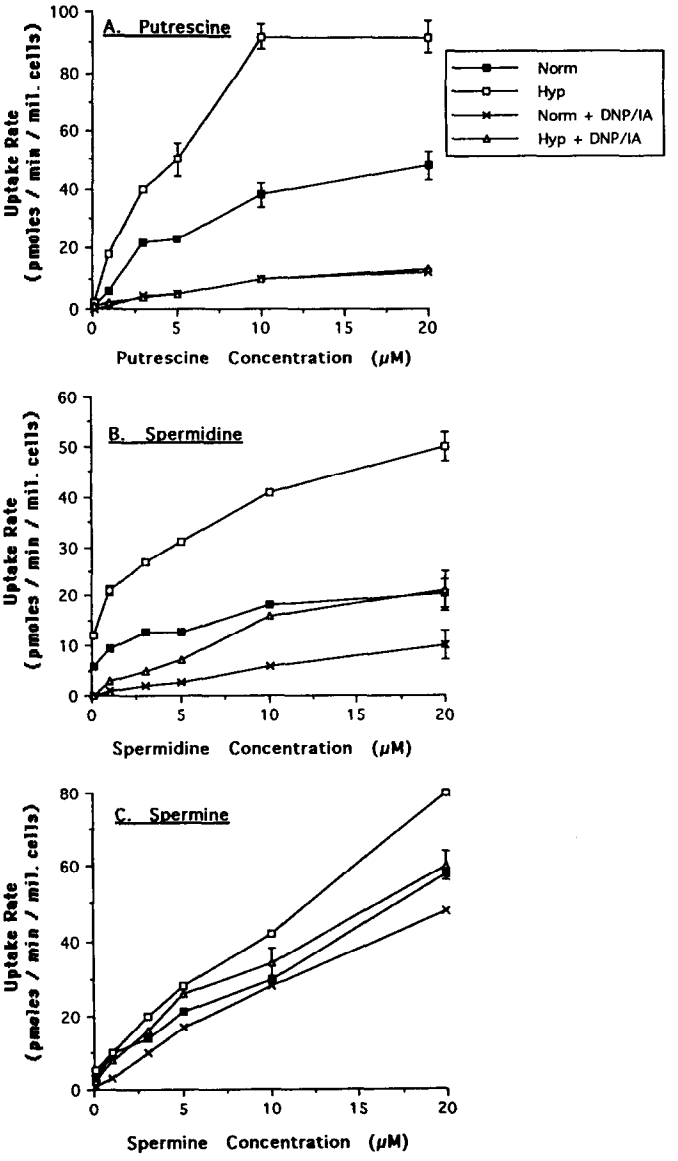

Fig. 1. Uptake rate–concentration curves for putrescine (A), spermidine (B), and spermine (C) in pulmonary artery smooth muscle cells cultured under standard (Po_2 > 100 torr) and hypoxic (Po_2 = 15–30 torr) conditions in the absence and presence of the metabolic inhibitors DNP (0.1 mM) and IA (1 mM). Each point is the mean ± SEM of at least six observations.

gradient on polyamine uptake. To further explore the role of Na^+ on the specific component of polyamine transport, increasing concentrations of several agents known to disrupt the Na^+ gradient were tested. Preincubation of PASMCs with ascending doses of ouabain (Fig. 4), a Na^+-K^+ pump inhibitor, had no effect on PUT transport in either hypoxic or standard PASMCs. On the other hand, SPD uptake was inhibited slightly by ouabain treatment in both standard and hypoxic cells. Ouabain was more effective against SPD uptake in hypoxic cells than standard cells at all concentrations tested. Ouabain failed to influence SPM transport in both standard and hypoxic cells (data not shown).

The effects of ionophores on the specific component of polyamine transport also were examined. As shown in Fig. 5, gramicidin, a Na^+ ionophore, reduced SPD uptake in hypoxic cells without altering transport in standard cells. These actions were not concentration-related. Gramicidin also suppressed PUT uptake in both standard and hypoxic cells, but only at the highest concentration examined (200 nM). Gramicidin (200 nM) also partially suppressed SPM transport in both standard and hypoxic cells (data not shown). These latter actions were again not related to the concentration of gramicidin, and the magnitude of inhibition did not exceed 20–30%, regardless of the specific

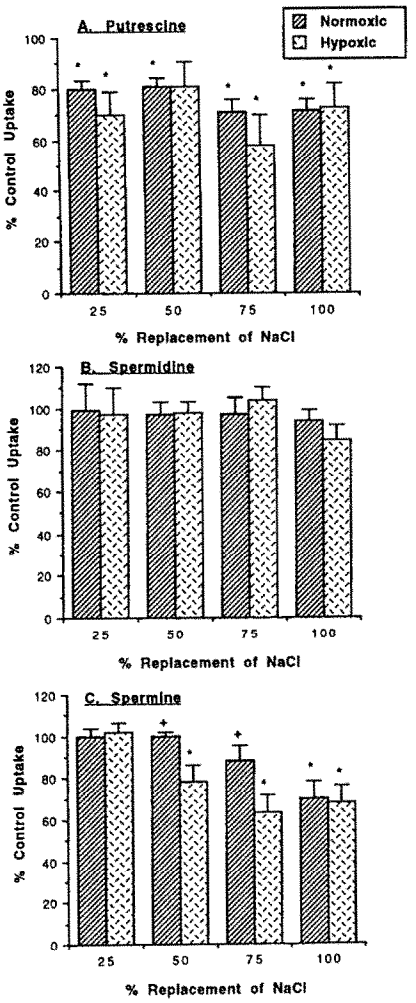

Fig. 2. Impact of graded replacement of Na^+ with choline on uptake of 3 μM putrescine (A), spermidine (B), and spermine (C) in pulmonary artery smooth muscle cells cultured under standard (Po_2 > 100 torr) or hypoxic (Po_2 = 15–30 torr) conditions. Key: (*) significantly different from control at $P < 0.05$, and (+) significantly different from hypoxic condition at $P < 0.05$. Each point is the mean ± SEM of at least six observations.

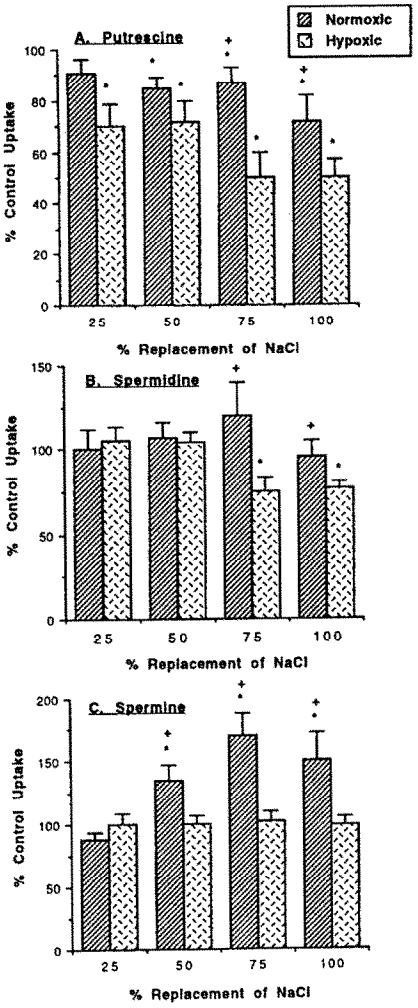

Fig. 3. Impact of graded replacement of Na^+ with sucrose on uptake of 3 μM putrescine (A), spermidine (B), and spermine (C) in pulmonary artery smooth muscle cells cultured under standard (Po_2 > 100 torr) or hypoxic (Po_2 = 15–30 torr) conditions. Key: (*) significantly different from control at $P < 0.05$, and (+) significantly different from hypoxic condition at $P < 0.05$. Each point is the mean ± SEM of at least six observations.

polyamine studied. Monensin, a Na^+/K^+ ionophore, was without effect on transport of PUT and SPD in either hypoxic or standard cells when added to the culture medium at a concentration of 1 μM (Fig. 6). At a 2 μM concentration, however, monensin dramatically increased uptake of PUT and SPD in cells cultured under standard conditions. The ionophore failed to alter uptake of these polyamines when cells were cultured under hypoxic conditions. In addition, monensin failed to influence the uptake of SPM in both standard and hypoxic cells at the two concentrations used in this experiment (data not shown).

DISCUSSION

Along with the capacity for *de novo* polyamine biosynthesis, cells also express a system(s) that transports polyamines from the extracellular to the intracellular compartment [1]. These two pathways, and perhaps others, interact to govern adjustments in cellular polyamine contents necessary for changes in phenotype or proliferative state. In the setting of hypoxic pulmonary hypertension, two general lines of evidence suggest that induction of polyamine transport plays a dominant role in driving lung cell responses underlying hypertensive remodeling of the pulmonary vasculature. First, the increased polyamine content noted in lungs from intact, chronically hypoxic rats is associated in time with depressed ODC activity and increased uptake and decreased efflux of putrescine [8]. Hypoxic PASMCs in culture also exhibit large decreases in ODC

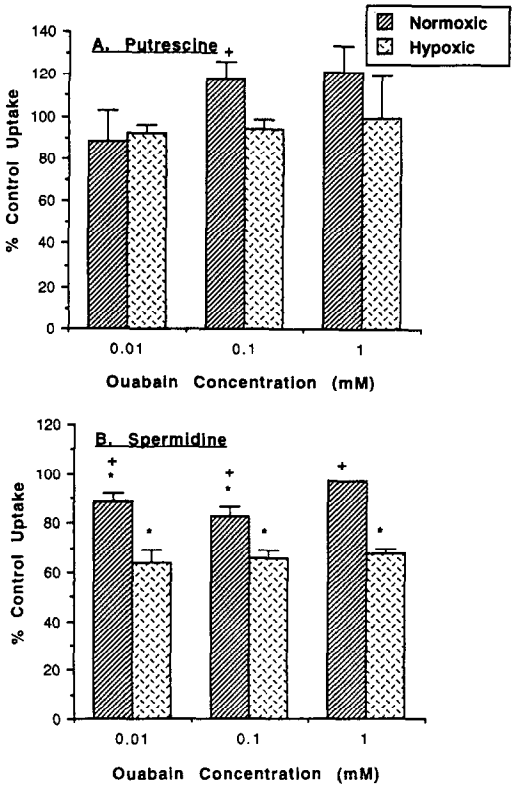

Fig. 4. Impact of ascending concentrations of ouabain, a Na^+-K^+ ATPase inhibitor, on uptake of 3 μM putrescine (A) and spermidine (B) in pulmonary artery smooth muscle cells cultured under standard ($Po_2 > 100$ torr) or hypoxic ($Po_2 = 15$–30 torr) conditions. Key: (*) significantly different from control at $P < 0.05$, and (+) significantly different from hypoxic condition at $P < 0.05$. Each point is the mean ± SEM of at least six observations.

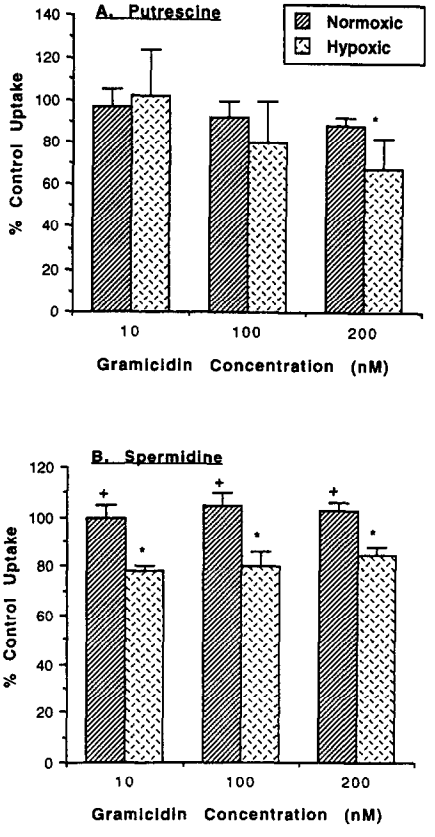

Fig. 5. Impact of the Na^+ ionophore gramicidin on uptake of 3 μM putrescine (A) and spermidine (B) in pulmonary artery smooth muscle cells cultured under standard ($Po_2 > 100$ torr) or hypoxic ($Po_2 = 15$–30 torr) conditions. Key: (*) significantly different from control at $P < 0.05$, and (+) significantly different from hypoxic condition at $P < 0.05$. Each point is the mean ± SEM of at least six observations.

mRNA abundance and activity, while increased uptake of all three polyamines can be attributed to induction of two transporters, a "non-selective" one that transports PUT, SPD, and SPM, and a "selective" pathway for SPD and SPM [9, 10]. In addition, hypoxic exposure seems to up-regulate both transporters through increasing the expression of new carriers. In the present study, we explored two likely energy sources for polyamine transport, ATP and the Na^+ gradient, as determinants of polyamine uptake rate in standard PASMCs and in cells wherein both putative polyamine transporters were induced by culture under hypoxic conditions. In this study, we determined whether both ATP and Na^+ play roles in the regulation of the two putative polyamine carriers in PASMCs. We were especially interested in the role of Na^+ in the induction of transport by hypoxia in light of previous reports showing that activation of the Na^+-dependent pathway in renal tubular cells was unmasked by a combination of epidermal growth factor and insulin but not other cell growth conditions [16].

Results of the present study indicate that operation of the two putative polyamine carriers in control and hypoxic PASMCs is attenuated by treatment with DNP + IA, agents that are well-known to inhibit ATP synthesis secondary to oxidative phosphorylation and glycolysis. The amounts of PUT, SPD and SPM uptake remaining in PASMCs treated with the metabolic inhibitors approximated those in cells incubated at 4°. A requirement for ATP synthesis as an energy source for polyamine transports has been reported for other cell types, including human umbilical vein endothelial cells [11], rat isolated hepatocytes [15], and murine mesenchymal cells [17].

Polyamine transport has been linked to the Na^+ electrochemical gradient. For example, it has been shown previously in type II pneumocytes that PUT and SPD, but not SPM, uptake was inhibited when extracellular NaCl was replaced iso-osmotically with ChCl [11]. Similar results were obtained for PUT uptake in murine embryonic palate mesenchymal cells [17]. To examine the role of the Na^+ gradient in PASMCs, we used a strategy somewhat more comprehensive than that employed in these previous

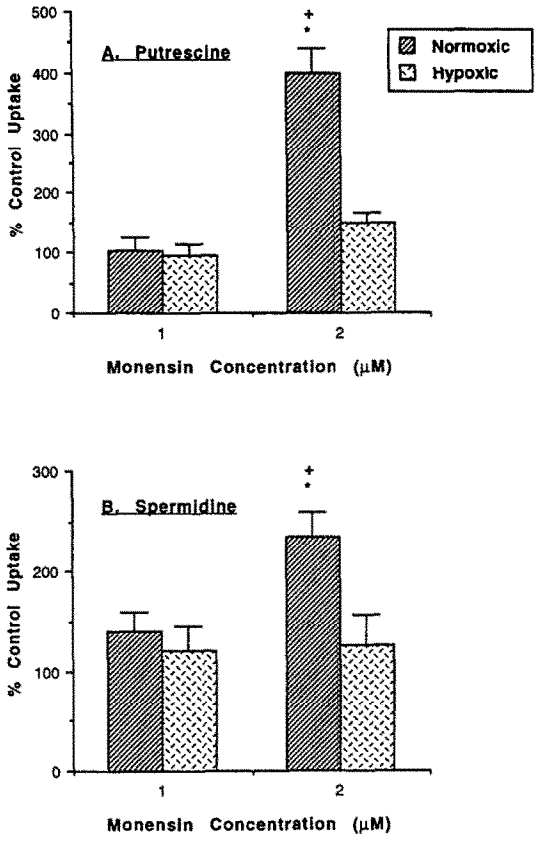

Fig. 6. Impact of the Na^+/K^+ ionophore monensin on uptake of 3 μM putrescine (A) and spermidine (B) in pulmonary artery smooth muscle cells cultured under standard ($Po_2 > 100$ torr) or hypoxic ($Po_2 = 15$–30 torr) conditions. Key: (*) significantly different from control at $P < 0.05$, and (+) significantly different from hypoxic condition at $P < 0.05$. Each point is the mean ± SEM of at least six observations.

reports. In brief, polyamine transport was assessed in the control state and when Na^+ was iso-osmotically replaced with the charged choline or the uncharged sucrose, when the Na^+ gradient was perturbed by inhibition of the Na^+-K^+ ATPase, and when Na^+ influx was enhanced by two ionophores with different mechanisms of action, gramicidin and monensin. Among the interventions tested, the most prominent effects were observed when NaCl was replaced by sucrose. Progressive replacement of the normal NaCl content with sucrose inhibited approximately 50% of PUT transport in hypoxic cells and about 30% in standard PASMCs. A similar trend was noted for SPD. In contrast, SPM uptake in standard but not hypoxic cells was increased by substitution of Na^+ with sucrose (data not shown). The effect of substituting Na^+ with ChCl on polyamine transport was similar although less impressive. The reduction in PUT and SPD uptake was greater in hypoxic cells than in standard cells; however, this difference was not statistically significant. These findings suggest that the two putative carriers in PASMCs and their up-regulation by hypoxia could have different properties. PUT uptake, for instance, is more sensitive to Na^+ replacement than SPD or SPM transport. Other data in the present investigation, however, do not support this suggestion.

Results of the above study also could suggest that the Na^+ gradient plays a partial role in polyamine transport regulation. This notion is supported by the findings that neither choline nor sucrose, even when completely replacing the normal Na^+ content, reduced transport of any of the polyamines by more than 50%, regardless of whether the cells were cultured under standard or hypoxic conditions. Polyamine uptake in a number of cell types has been reported to be at least partially Na^+ dependent [11–13, 16]. One possible explanation for partial Na^+ dependency could be attributed to the presence of two components for polyamine transport, one Na^+ dependent and other Na^+ independent. In addition, the transporters operative in hypoxic cells may differ from standard PASMCs with respect to the contribution of the Na^+ gradient. This conclusion is also supported by the evidence of the present investigation.

Although the effects of other interventions on polyamine transport were inconsistent with regard to Na^+ replacement studies, they seem to support the suggestion that polyamine transport is partially dependent on Na^+. For example, ouabain, an inhibitor of the Na^+-K^+ ATPase, failed to influence PUT transport in either standard or hypoxic cells, but it significantly reduced SPD transport in both standard and hypoxic cells. Ouabain was also more effective in reducing SPD transport in hypoxic cells than in standard cells. The actions of the ionophores also were inconsistent. The Na^+ ionophore, gramicidin, partially impaired PUT transport in both standard and hypoxic cells. Gramicidin attenuated PUT transport in hypoxic cells more than in standard cells. SPD uptake, on the other hand, was partially reduced in hypoxic cells, but not in standard cells. The extent of inhibition did not exceed 20%, regardless of the gramicidin concentration. Monensin, a Na^+/K^+ ionophore, markedly increased PUT and SPD uptake, but only in standard cells, while SPM transport was unaffected. It is appreciated that the pharmacological agents used in this study do not selectively alter the Na^+ gradient; the disposition of other ions and perhaps other signaling pathways also may be affected. Nevertheless, the disparate and unimpressive actions of these agents, whose unifying pharmacologic effect is modulation of the Na^+ gradient, indicate that if Na^+ does play a role in driving polyamine transport in standard and hypoxic PASMCs, its contribution is partial and the mechanism is complicated. In addition, although the magnitude of the reduction in the polyamine transport system after Na^+ manipulation is partial, this effect is more impressive in hypoxic cells than in standard cells. This may suggest an induction of Na^+-dependent polyamine carriers by hypoxia. Further studies are necessary to characterize and discriminate between both Na^+-dependent and Na^+-independent components of polyamine uptake and, more importantly, to study the interaction between

the Na^+-dependent component and the induction of the two polyamine transporters by hypoxia.

In summary, in agreement with our previous report [10], hypoxic exposure up-regulates polyamine transport presumably through the expression of new carriers. In addition, results of the present study indicate that both constitutive polyamine transport in standard PASMCs and elevated transport in hypoxic cells require ATP synthesis. Data also suggest that a relatively minor component of the polyamine transport in PASMCs is Na^+ dependent, and that hypoxic exposure may promote the induction of Na^+-dependent polyamine carriers.

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