ATP-dependent K^+ Channels Modulate Vasoconstrictor Responses to Severe Hypoxia in Isolated Ferret Lungs

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Abstract

In normo- and hypoglycemic ferret lungs, the pulmonary vascular response to severe hypoxia ($PiO_2 \leq 10$ mmHg) is characterized by an initial intense vasoconstriction followed by marked vasodilation, whereas in hyperglycemic lungs, vasodilation is minimal, causing vasoconstriction to be sustained. In contrast, the response to moderate hypoxia is characterized by a slowly developing sustained vasoconstriction which is unaffected by glucose concentration. To determine the role of ATP-dependent K^+ (K_{ATP}) channels in these responses, we examined the effects of cromakalim, which opens K_{ATP} channels, and glibenclamide, which closes them. During steady-state vasoconstriction induced in isolated ferret lungs by moderate hypoxia, cromakalim caused dose-dependent vasodilation ($EC_{50} = 7 \times 10^{-7}$ M) which was reversed by glibenclamide ($IC_{50} = 8 \times 10^{-7}$ M), indicating that K_{ATP} channels were present and capable of modulating vascular tone. During severe hypoxia in hypoglycemic lungs ([glucose] < 1 mM), glibenclamide markedly inhibited the secondary vasodilation. Raising perfusate glucose concentration to 14±0.4 mM had the same effect. As a result, initial vasoconstrictor responses were well sustained. However, neither glibenclamide nor hyperglycemia affected vasoconstrictor responses to moderate hypoxia or KCl, indicating that effects during severe hypoxia were not due to nonspecific potentiation of vasoconstriction. These findings suggest that in the ferret lung (*a*) severe hypoxia decreased ATP concentration and thereby opened K_{ATP} channels, resulting in increased K^+ efflux, hyperpolarization, vasodilation, and reversal of the initial vasoconstrictor response; and (*b*) hyperglycemia prevented this sequence of events. (*J. Clin. Invest.* 1991. 88:500–504.) Key words: sulfonylurea • glibenclamide • cromakalim • pulmonary vasoconstriction • pulmonary vascular resistance • hypoxia

Introduction

In isolated lungs of several species, the pulmonary vascular response to inspired oxygen tensions ≤ 10 mmHg is characterized by an initial intense vasoconstriction, followed by marked vasodilation (1–3). In ferret lungs this vasodilation is inhibited by high glucose concentrations (15 mM) (3), suggesting that severe hypoxia limited some component of the vasoconstrictor response, perhaps by decreasing production of ATP. It is unlikely, however, that this ATP-dependent component was the contractile apparatus of vascular smooth muscle, because neither severe hypoxia nor glucose concentration affected pulmonary vasoconstrictor responses to KCl (3, 4).

Recently, ATP-dependent K^+ (K_{ATP})[1] channels were described in myocardial cells (5), vascular smooth muscle (6–10), pancreatic beta-cells (11, 12), and neurons (13). These channels are thought to mediate cellular responses to changes in glucose and/or oxygen supply (10, 13, 14). For example, when ATP concentration was decreased, K_{ATP} channels opened, causing increased K^+ efflux and hyperpolarization (9, 14). In vascular smooth muscle, this sequence of events caused vasodilation (6–10). It is now appreciated that K_{ATP} channels can be opened (activated) by compounds such as diazoxide or cromakalim, and closed (inactivated) by sulfonylureas such as tolbutamide and glibenclamide (6, 14, 15).

In this study, we hypothesized that the inability of pulmonary vessels to sustain vasoconstriction during severe hypoxia was caused by the opening of K_{ATP} channels, and that hyperglycemia reversed this inability by increasing ATP production sufficiently to keep these channels closed. To test these hypotheses, we determined the effects of cromakalim and glibenclamide on hypoxic vasomotor responses of isolated ferret lungs exposed to high and low perfusate glucose concentrations.

Methods

Preparation. Adult male ferrets (1,100–1,900 g) were anesthetized with intraperitoneal sodium pentobarbital (20–40 mg/kg) and ventilated with 30% O_2 at a tidal volume of 12.5 ml/kg, a frequency of 10–12/min, and an end-expiratory pressure of 3–4 mmHg with a volume cycled ventilator (model 665; Harvard Apparatus, South Natick, MA). The abdomen was opened with a midline incision and heparin (1,000 U) was injected into the inferior vena cava. The animals were exsanguinated through a catheter placed in the abdominal aorta. After exsanguination, 5.0–5.4% CO_2 was added to the inspired gas. The chest was opened at the midline and a metal cannula was placed in the main pulmonary artery. The left atrium was drained into a reservoir through a large plastic cannula inserted through the left ventricle. Using a peristaltic pump (Minipuls 2; Gilson Co., Inc. Middleton, WI), the isolated lungs were perfused at 100 ml · min^{-1} · kg^{-1} through a bubble filter with ~ 50 ml of autologous blood mixed with ~ 50 ml of 3% Dextran-70 in lactated Ringer's solution (perfusate hematocrit 20–25%). Left atrial pressure was < 0 mmHg. The time from exsanguination to perfusion was less than 30 min.

Pulmonary arterial, left atrial, and tracheal pressures were measured continuously with pressure transducers (model P10EZ; Spectramed Inc., Oxnard, CA) referenced to the bottom of the lungs, and recorded on a chart recorder (model 7E; Grass Instr. Co., Quincy, MA). Perfusate temperature, measured with a thermistor placed in the left atrial effluent, was kept at 39–40°C by immersing the reservoir and

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1. *Abbreviations used in this paper:* K_{ATP}, ATP-dependent K^+; Ppa, pulmonary arterial pressure.

extracorporeal tubing in a heated water bath. Inspired O_2 (PiO_2) and CO_2 ($PiCO_2$) tensions were measured (Gas Analyzers OM-11 and LB-2; Sensormedics Corp., Anaheim, CA) at the gas intake port of the ventilator and recorded continuously. After initiation of perfusion, PiO_2 was 200 mmHg and later varied according to protocol. $PiCO_2$ was constant at 36–39 mmHg. Left atrial blood samples were collected periodically for measurement of blood gas tensions and pH (Radiometer America, Inc., Cleveland, OH). Perfusate pH was kept at 7.35–7.45 by the addition of small amounts of 1N $NaHCO_3$ as needed. Vascular responses were measured continuously as mean end-expiratory pulmonary arterial pressure at the constant flow of 100 $ml \cdot min^{-1} \cdot kg^{-1}$ (Ppa_{100}). Maximal vasoconstrictor responses were calculated as the difference between peak Ppa_{100} and baseline Ppa_{100}, whereas sustained vasoconstrictor responses were calculated as the difference between steady-state Ppa_{100} and baseline Ppa_{100}. The vasodilator phase of the response to anoxia was quantified by subtracting nadir Ppa_{100} from peak Ppa_{100}.

Perfusate glucose concentrations were monitored using a spectrophotometric method. A 1-ml sample of perfusate was centrifuged at 750 *g* for 6 min and 10 μl of supernatant was mixed with 1.5 ml of hexokinase reagent (Sigma Chemical Co., St. Louis, MO). After 10 min, absorbance was measured at 340 nm. Perfusate glucose concentrations fell spontaneously to < 1 mM at 180 min of perfusion unless glucose was added to the perfusate.

Effects of cromakalim and glibenclamide on the response to moderate hypoxia. To determine if K_{ATP} channels were present and modulated pulmonary vascular tone, we measured the effects of cromakalim during sustained vasoconstrictor responses to moderate hypoxia in three lungs. After 30 min of perfusion the lungs were ventilated with 4.3% O_2–5.4% CO_2 (PiO_2 = 30 mmHg). When Ppa_{100} was stable (25–30 min), cromakalim was added to the perfusate in amounts calculated to achieve final concentrations of 10^{-8}, 3×10^{-8}, 10^{-7}, 3×10^{-7}, 10^{-6}, and 3×10^{-6} M. Adequate time was allowed after each dose of cromakalim for Ppa_{100} to achieve a new steady-state.

If cromakalim-induced vasodilation during hypoxia were caused by opening K_{ATP} channels, closing the channels should reverse the vasodilation. To test this possibility, glibenclamide was added to the perfusate after administration of the maximal dose (3×10^{-6} M) of cromakalim to the three hypoxic lungs in amounts calculated to achieve final concentrations of 10^{-7}, 3×10^{-7}, 10^{-6}, and 3×10^{-6} M. After each dose of glibenclamide, adequate time was allowed for Ppa_{100} to reach a new steady-state.

Effects of glucose and glibenclamide on the response to anoxia. To generate vasomotor responses to anoxia, the inspired oxygen tension at the intake port of the ventilator was decreased from 200 to 0 mmHg in a square-wave fashion at 60, 120, and 180 min of perfusion. After 30 min, PiO_2 was returned to 200 mmHg.

To determine the effects of high glucose concentration on the anoxic response, we added either 15 mM glucose (n = 5) or 15 mM sucrose (n = 5) to the perfusate 15 min before the third anoxic exposure. This concentration of glucose was chosen because we had shown previously that it allowed vasoconstriction during severe hypoxia to be sustained (3). Sucrose, a disaccharide that is neither taken up nor metabolized, was used to control for possible osmotic effects of glucose.

To determine if the effects of high glucose concentration on the response to anoxia were due to an effect on K_{ATP} channels, we added either 5 μM glibenclamide plus 15 mM sucrose (n = 5), or glibenclamide vehicle (methanol) plus 15 mM sucrose (n = 5) to the perfusate 15 min before the third anoxic exposure (180 min). This concentration of glibenclamide was chosen on the basis of the concentration-response experiments described above in order to achieve complete inhibition of the K_{ATP} channels.

Effects of glibenclamide on the response to moderate hypoxia. We found previously that hyperglycemia did not influence the pulmonary vascular response to moderate hypoxia (PiO_2 = 30 mmHg) in isolated ferret lungs (3). If our hypotheses were correct, glibenclamide also should have no effect on the vascular response to moderate hypoxia. Therefore, we exposed 10 lungs to PiO_2 = 30 mmHg for 30 min at 60, 120, and 180 min of perfusion. 15 min before the third hypoxic exposure, we added either 5 μM glibenclamide plus 15 mM glucose (n = 5) or glibenclamide vehicle plus 15 mM glucose (n = 5) to the perfusate.

Effects of glibenclamide on the response to KCl. To determine whether glibenclamide affected the ability of pulmonary vascular smooth muscle to contract in a nonspecific fashion, vasoconstrictor responses to KCl were measured in hyperglycemic lungs perfused with blood containing 5 μM glibenclamide (n = 4) or vehicle (n = 4). The lungs were exposed to 30 min of moderate hypoxia at 60, 120, and 180 min, as described above. After completion of the third hypoxic exposure when PiO_2 = 200 mmHg, KCl (1 M) was added to the perfusate, over 1–2 min, in amounts sufficient to achieve increases in perfusate potassium concentration of 10, 20, and 40 mM. At each concentration, 5–10 min were allowed for Ppa_{100} to achieve a stable response. Ventilation was stopped and the trachea occluded just before administration of KCl to avoid secondary influences of bronchospasm on pulmonary vascular resistance.

Preparation of solutions. Glibenclamide (Sigma Chemical Co.) and cromakalim (Smith, Kline, and Beecham; King of Prussia, PA) were prepared before each experiment as 1-mM stock solutions. Glibenclamide was dissolved in methanol and cromakalim in distilled water. Glucose was added as a 2.8 M solution in water, and sucrose as a 1.5 M solution in water.

Statistical analysis. Results were analyzed using two factor (perfusate constituent, time) split-plot analysis of variance (16). When a significant interaction was present, the least significant difference was calculated to allow comparison of means at each time point. Differences were considered significant when $P < 0.05$. Values in the text are means±SE.

Results

The pulmonary vascular response to increasing concentrations of cromakalim during moderate hypoxia is shown in Fig. 1 *a*. Exposure to a PiO_2 of 30 mmHg raised Ppa_{100} from 13±0.5 to 45±2.0 mmHg ($P < 0.05$). Cromakalim caused a concentration-dependent decrease in Ppa_{100} at concentrations $\geq 3 \times 10^{-8}$, with an EC_{50} of 7×10^{-7} M. The maximal concentration of cromakalim (3×10^{-6} M) reduced Ppa_{100} to 15±0.5 mmHg.

As shown in Fig. 1 *b*, the vasodilation induced by 3×10^{-6} M cromakalim was progressively reversed by glibenclamide at concentrations > 10^{-7} M, with an IC_{50} of 8×10^{-7} M. The

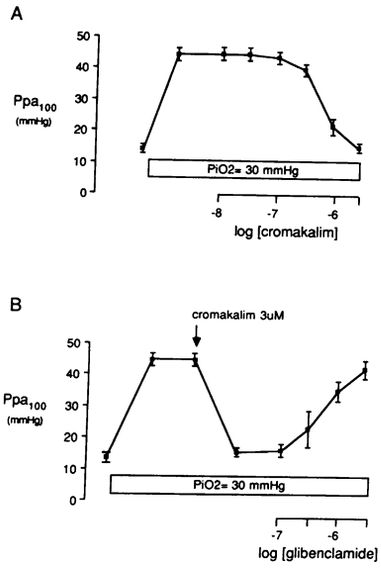

Figure 1. (*A*) Effect of cromakalim on the pulmonary vascular response to moderate hypoxia (n = 3). Hypoxia caused vasoconstriction which was reversed by cromakalim in a dose-dependent fashion. (*B*) Effect of glibenclamide on cromakalim-induced vasodilation during moderate hypoxia (n = 3). Glibenclamide reversed the effects of 3 μM cromakalim in a dose-dependent fashion. PiO_2, inspired oxygen tension.

maximum concentration of glibenclamide (3×10^{-6} M) caused a 90% reversal of cromakalim-induced vasodilation.

The effects of glucose on the vascular response to anoxia are shown in Fig. 2 *a*. Perfusate glucose concentrations averaged 14±0.4 mM in glucose lungs, and 0.4±0.1 mM in sucrose lungs at the beginning of the third anoxic exposure (180 min of perfusion). Glucose and sucrose lungs exhibited an intense, early vasoconstriction which was maximum (ΔPpa_{100} = 41±2.5 mmHg) at 4 min of anoxia. Subsequently, the sucrose lungs exhibited a rapid vasodilation (ΔPpa_{100} = −42±3.4 mmHg) to baseline levels, followed by a smaller, late vasoconstriction (ΔPpa_{100} = 6.6±1.3 mmHg). In contrast, glucose lungs had significantly less vasodilation (ΔPpa_{100} = −19±2.7 mmHg) and a greater late sustained vasoconstriction (ΔPpa_{100} = 22±1.2 mmHg). Vasomotor responses to anoxia did not differ between glucose and sucrose lungs in the first (60 min of perfusion) and second (120 min of perfusion) anoxic exposures.

The effects of glibenclamide on the vascular response to anoxia are shown in Fig. 2 *b*. The glucose concentration in these lungs averaged 0.3±0.1 mM at the beginning of the third anoxic exposure. Both groups of lungs had an intense early vasoconstriction. There was no statistical difference in the magnitude of peak vasoconstrictor responses, but peak responses occurred later in the glibenclamide lungs. For example, in the glibenclamide lungs, vasoconstriction was maximum (ΔPpa_{100} = 54±6.5 mmHg) at 5.5±0.4 min of anoxia, whereas in vehicle lungs, vasoconstriction was maximum (ΔPpa_{100} = 46±2.5 mmHg) at 3.5±0.2 min of anoxia. Vehicle lungs exhibited the same marked vasodilation (ΔPpa_{100} = −47±2.0 mmHg) and slight late vasoconstriction (ΔPpa_{100} = 11±3.6 mmHg) observed in sucrose lungs (Fig. 2 *a*). In contrast, glibenclamide lungs had less vasodilation (ΔPpa_{100} = −24±4.4 mmHg) and a greater sustained vasoconstriction (ΔPpa_{100} = 30±2.7 mmHg). Thus, the effects of glibenclamide on the vascular response to anoxia (Fig. 2 *b*) were similar to the effects of glucose (Fig. 2 *a*). There were no differences between glibenclamide and vehicle lungs in the vasomotor responses to the first and second anoxic exposures, which were performed before the addition of glibenclamide or vehicle.

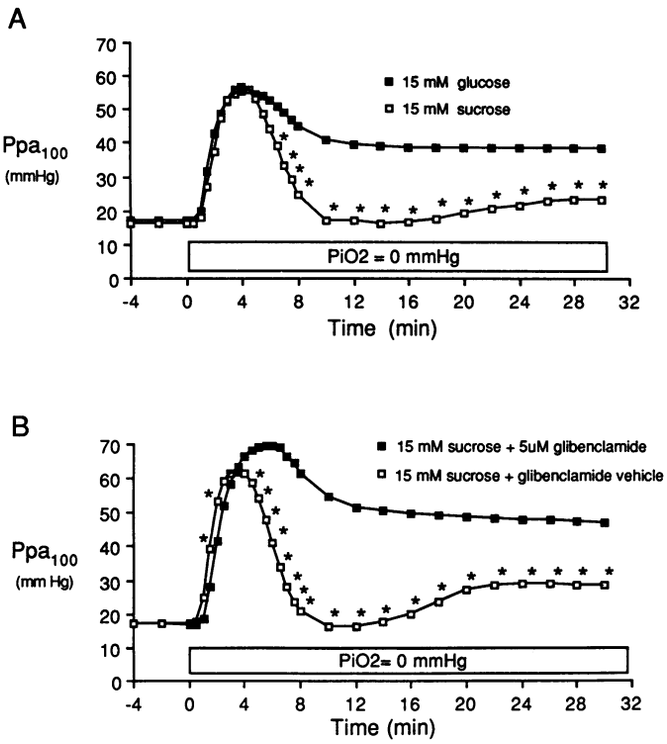

Figure 2. (*A*) Effect of high glucose concentration on the pulmonary vascular response to anoxia. 15 mM glucose (n = 5) or sucrose (n = 5) was added to the perfusate 15 min before the anoxic exposure at 180 min of perfusion. (*B*) Effect of glibenclamide (5 μM) on the pulmonary vascular response to anoxia. Sucrose plus glibenclamide (n = 5) or sucrose plus glibenclamide vehicle (n = 5) was added to the perfusate 15 min before the anoxic exposure at 180 min of perfusion. PiO_2, inspired oxygen tension; asterisks indicate significant differences ($P < 0.05$) between the groups.

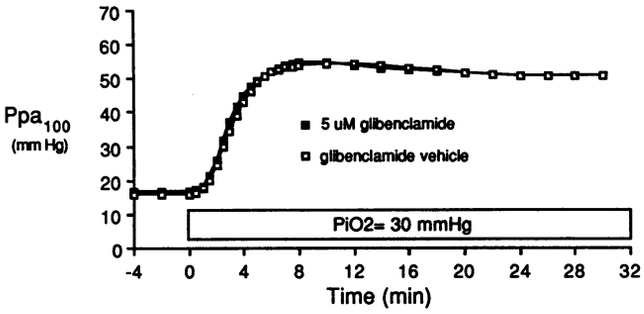

Figure 3. Effect of glibenclamide on the pulmonary vascular response to moderate hypoxia. Glibenclamide (n = 5) or glibenclamide vehicle (n = 5) was added to lungs perfused with 15 mM glucose 15 min before the hypoxic exposure at 180 min of perfusion.

As shown in Fig. 3, glibenclamide had no effect on vasoconstrictor responses to moderate hypoxia (PiO_2 = 30 mmHg). In contrast to anoxia, moderate hypoxia caused a slowly developing vasoconstriction (ΔPpa_{100} = 39±2.5 mmHg) which peaked at 8.5±0.6 min and was followed by a slight vasodilation (ΔPpa_{100} = −4.6±0.9 mmHg).

Fig. 4 demonstrates that KCl caused concentration-dependent increases in Ppa_{100}. Glibenclamide (5×10^{-6} M) did not alter vasoconstrictor responses to KCl.

Discussion

The vasomotor response to anoxia in hypoglycemic ferret lungs was characterized by an intense early vasoconstriction followed by a marked vasodilation to baseline and finally a slight late vasoconstriction (Fig. 2 *a*). Increasing perfusate glucose concentration to 14±0.4 mM (Fig. 2 *a*) significantly reduced the vasodilation and increased the sustained vasocon-

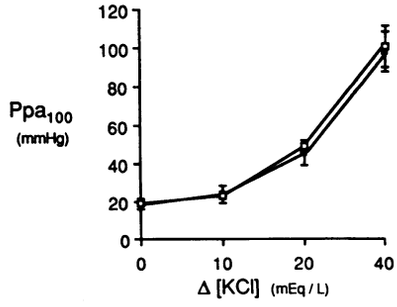

Figure 4. Effect of glibenclamide on pulmonary vascular response to KCl. KCl was added to lungs containing glibenclamide (5 μm) (■) (n = 4) or glibenclamide vehicle (□) (n = 4), and 5–10 min were allowed at each concentration of KCl to achieve a stable response.

striction. These results confirm our previous observations, which also demonstrated that the effects of high glucose concentration were not reproduced by normal (5 mM) glucose concentration (3).

The cells best known for their ability to respond to high glucose concentrations are pancreatic beta cells, which release insulin. The mechanism of this response is thought to involve K_{ATP} channels in the plasma membrane of the beta cell (11, 12, 14). When glucose concentration is high, ATP production by these cells is increased. The resultant increase in ATP concentration causes closure of the K_{ATP} channels, leading to depolarization and secondary release of insulin. Sulfonylureas, such as glibenclamide and tolbutamide, are thought to cause insulin release from these cells by closing K_{ATP} channels (14, 17). Presumably, this is the basis for the therapeutic efficacy of these agents in patients with adult-onset (Type II) diabetes mellitus.

High glucose concentration was also found to stimulate neuronal cells of the substantia nigra (13). In this case, stimulation resulted in release of gammaaminobutyric acid (GABA). Because this response was also elicited by sulfonylureas and associated with decreased efflux of Rb^+ (13), a K^+ analogue (14), it was thought to result from closure of K_{ATP} channels. Furthermore, when ATP concentration was reduced by anoxia, Rb^+ efflux increased and GABA secretion was inhibited. Because the effects of anoxia were blocked by high glucose concentrations or sulfonylureas, they were also thought to be due to opening of K_{ATP} channels (13).

The results of these studies (11–13) suggested that hypoglycemic lungs exposed to anoxia might have been unable to sustain hypoxic vasoconstriction (Fig. 2 *a*) because deficient ATP production caused opening of K_{ATP} channels, K^+ efflux, hyperpolarization, and vasodilation. Furthermore, analogous to its effects in substantia nigra neurons, high glucose concentration may have allowed vasoconstriction to be sustained during anoxia by maintaining ATP concentrations at levels sufficient to keep K_{ATP} channels closed. In this study, we tested these possibilities by using the pharmacologic agents, cromakalim and glibenclamide.

Cromakalim is thought to open K_{ATP} channels (15). For example, in vascular smooth muscle, cromakalim caused relaxation in association with hyperpolarization and increased Rb^+ efflux (6, 7, 9). These effects seemed to be relatively specific because single channel recordings demonstrated that cromakalim did not open Ca^{++}-activated K^+ channels in smooth muscle (6), and because inhibitors of Ca^{++}-dependent K^+ channels, such as charybdotoxin and apamin, did not block the effects of cromakalim in portal vein (7). The EC_{50} of cromakalim's activation of K_{ATP} channels in systemic vascular smooth muscle was estimated to be 0.05–0.2 μM (7–9).

As stated above, glibenclamide is thought to close K_{ATP} channels (6, 8, 10, 13, 14, 17). For example, in rat aortic rings contracted with KCl, glibenclamide (0.1–3 μM) had no effect on resting tension and inhibited cromakalim-induced relaxation, but did not inhibit relaxation induced by the calcium channel blockers, diltiazem and nitrendipine (8). Single channel recordings of arterial smooth muscle cells also demonstrated that K_{ATP} channels were activated by cromakalim (1 μM) and inactivated by the addition of glibenclamide (20 μM) (6). As mentioned above, in substantia nigra neuronal cells, the increased Rb^+ efflux caused by anoxia or depletion of ATP was reversed by glibenclamide (13). Glibenclamide has not been demonstrated to block any other type of K^+ channel in smooth muscle or pancreatic cells (6, 14). The EC_{50} for these effects of glibenclamide varied between 0.1 and 10 μM (8, 9, 13).

Our first experiment was designed to determine the effects of cromakalim and glibenclamide on pulmonary vessels during hypoxia-induced vasoconstriction. As shown in Fig. 1 *a*, pulmonary vasoconstriction induced by moderate hypoxia was reversed by cromakalim at concentrations $\geq 3 \times 10^{-8}$ M with an EC_{50} of ~ 0.7 μM. These concentrations are similar to those shown previously to open K_{ATP} channels (6–10). The vasodilation induced by cromakalim was reversed by glibenclamide at concentrations $\geq 3 \times 10^{-7}$ M (Fig. 1 *b*), with an IC_{50} of ~ 0.8 μM. These concentrations are similar to those shown previously to close K_{ATP} channels (6, 8, 9). Thus, these results suggested that K_{ATP} channels were present in the lung and capable of modulating pulmonary vasomotor tone during hypoxia. Additional evidence that these channels modulated vascular tone in lung was recently obtained by Yuan et al. (18) and Eltze (19) who demonstrated that cromakalim inhibited hypoxia- and KCl-induced contractions in isolated pulmonary arteries, and that glibenclamide competitively antagonized the vasorelaxant effects of cromakalim.

Our second experiment was performed to determine if the effects of glibenclamide on the response to anoxia were similar to those of high glucose concentration. As shown in Fig. 2 *b*, glibenclamide markedly inhibited the vasodilator phase of the anoxic response, allowing the initial vasoconstriction to be better sustained in the absence of glucose. These results were similar to those of high glucose concentration (Fig. 2 *a*), and are consistent with the hypothesis that high glucose concentration allowed vasoconstriction to be sustained during anoxia because it maintained ATP production and closure of K_{ATP} channels.

To determine if the effects of glibenclamide were due to nonspecific potentiation of vasoconstriction, we determined its effects on pulmonary vasoconstrictor responses to moderate hypoxia or KCl, which we previously demonstrated to be unaffected by glucose (3). As shown in Figs. 3 and 4, glibenclamide did not affect the vasoconstrictor response to either moderate hypoxia or KCl, suggesting that K_{ATP} channels did not modulate vasoconstriction under these conditions. In these experiments, the effects of glibenclamide again resembled those of high glucose concentrations (3). Thus, neither glibenclamide nor hyperglycemia appeared to alter the anoxic response through a nonspecific potentiation of vasoconstriction.

Recently, it was concluded that glibenclamide acted as a competitive antagonist of the thromboxane receptor in canine coronary artery (20). It is not clear how this action, if present in our experiments, could explain our results, because thromboxane causes pulmonary vasoconstriction (21), and inhibition of thromboxane and prostaglandin synthesis by indomethacin did not prevent reversal of hypoxic pulmonary vasoconstriction during severe hypoxia (22).

Our results are compatible with those of Daut and colleagues (10), who found that coronary vasodilation induced in isolated perfused guinea pig hearts by anoxia, dinitrophenol, cyanide, or cromakalim was inhibited by glibenclamide, whereas vasodilation caused by bradykinin was unaffected. They concluded that coronary arteries dilated in response to anoxia because depletion of ATP caused activation of K_{ATP} channels and secondary smooth muscle relaxation. The isolated heart differed from the isolated lung in that anoxic vasodilation in the coronary vasculature was not preceded by transient vasoconstriction. Thus, while the pulmonary vasculature

characteristically constricts in response to hypoxia, during severe hypoxia it appears to share a mechanism of vasodilation with the coronary vasculature.

Our results are inconsistent with those of Robertson and colleagues (23), who found, in isolated rat lungs, that high concentrations of tolbutamide (1.7×10^{-4}–8.5×10^{-3} M) inhibited or abolished vasoconstrictor responses of isolated rat lungs to 2% O_2, whereas high concentrations of diazoxide (1.1×10^{-3} M) caused vasoconstriction during normoxia. Because tolbutamide, like glibenclamide, is thought to close K_{ATP} channels (14), and diazoxide, like cromakalim, is thought to open them (24), the authors speculated that hypoxia activated K_{ATP} channels in cells other than smooth muscle, resulting in hyperpolarization and secondary release of factors that caused vasoconstriction. We cannot explain the discrepancies in results other than to suggest that at high concentrations, tolbutamide and diazoxide could have nonspecific effects or that the effects of these agents differ among species.

As shown in Figs. 2 *B* and 3, glibenclamide did not cause vasoconstriction during normoxia, suggesting that K_{ATP} channels were closed during baseline conditions. This observation, which has also been made in pulmonary and systemic vascular smooth muscle, differs from results in the pancreatic beta-cell, where a proportion of the channels are thought to be open under normoxic, normoglycemic conditions (8, 9, 11, 14, 18, 19).

Our data provide no insight concerning the location of K_{ATP} channels apparently involved in modulation of the response to anoxia. Although these channels are found in vascular smooth muscle (6), it is possible that the vasodilation phase of the anoxic response (Fig. 2 *a*) was due to activation of K_{ATP} channels in some other cell. One obvious possibility is endothelium, which is stimulated by hyperpolarization (25) and can release vasodilators such as endothelium-derived hyperpolarization factor (EDHF), which induces relaxation in association with increased Rb^+ efflux in smooth muscle cells (26).

In summary, our results indicate that (*a*) K_{ATP} channels were present in the isolated ferret lung and were capable of modulating pulmonary vasomotor tone, and (*b*) glibenclamide, like high glucose concentrations, permitted hypoxic vasoconstriction to be sustained during anoxia, but did not affect vasoconstrictor responses to moderate hypoxia or KCl. These results support the hypothesis that hypoxic vasoconstriction is not sustained during anoxia due to opening of K_{ATP} channels, and that high glucose concentrations increase ATP production sufficiently to maintain channel closure, thereby preventing K^+ efflux, hyperpolarization, and vasodilation. Our data added to the growing evidence that K_{ATP} channels play an important role in modulating blood flow during periods of deprivation of substrates for ATP production (27).

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