Delayed rectifier potassium channels contribute to the depressed pulmonary artery contractility in pneumonia

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Yaghi, Asma, Sanjay Mehta, and David G. McCormack. Delayed rectifier potassium channels contribute to the depressed pulmonary artery contractility in pneumonia. J Appl Physiol 93: 957–965, 2002. First published June 7, 2002; 10.1152/japplphysiol.01146.2001.—We investigated the role of K+ channels in the attenuated pulmonary artery (PA) contractility characteristic of acute Pseudomonas pneumonia. Contractility of PA rings from the lungs of control or pneumonia rats was assessed in vitro by obtaining cumulative concentration-response curves to the contractile agonists KCl, phenylephrine, or PGF2α. However, 4-aminopyridine (2 mM), a blocker of voltage-gated K+ channels (delayed rectifier K+ channel blocker), 80 μM had no significant effect on the attenuated contractile responses to KCl, phenylephrine, and PGF2α. Therefore, large-conductance Ca2+-activated K+ channels, and glybenclamide (ATP-sensitive K+ channel blocker, 80 μM) had no significant effect on the attenuated contractility observed in this model of acute pneumonia. In contrast, 4-aminopyridine enhances contraction in PA rings from pneumonia lungs, consistent with involvement of a voltage-gated K+ channel in the depressed PA contractility in acute pneumonia. Unraveling the precise mechanism of attenuated contractility in pneumonia could lead to innovative therapies for the pulmonary vascular abnormalities associated with this disease.

4-aminopyridine; glybenclamide; levromakalim; rat intrapulmonary artery; Pseudomonas pneumonia

Our laboratory has previously demonstrated, both in vitro (55) and in vivo (19), depressed contractility of the pulmonary vasculature in rats with Pseudomonas pneumonia. Excess nitric oxide (NO) (produced by inducible NO synthase) partly contributes, but does not fully explain, the depressed pulmonary vascular contractility observed in rats with acute pneumonia (55). In addition, our laboratory has suggested a possible role for cytochrome P-450 (CYP) metabolites [epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE)] of arachidonic acid (AA) in this phenomenon (57). Therefore, the mechanism of action involved in this phenomenon is not yet completely understood, although K+ channel activation may contribute to endotoxin-mediated hyporeactivity to vasoconstrictor agents in some vascular beds (9, 22, 35).

Many endogenous and synthetic agents can modulate the contractility or relaxation of vascular beds and are used to investigate vascular reactivity in vivo and in vitro. Some of these agents are constrictors, such as phenylephrine (PE) and PGF2α, whereas others are dilators, such as acetylcholine, sodium nitroprusside, and PGI2 (17, 45, 51). In acute lung injury (such as occurs in pneumonia or systemic sepsis), attenuated pulmonary vascular contractility has been demonstrated both in vivo (18–20) and in vitro (31, 53, 56) to a number of stimuli, including hypoxia, angiotensin II, norepinephrine, and potassium chloride (KCl). Furthermore, sepsis and pneumonia impair hypoxic pulmonary vasoconstriction, thereby reducing arterial oxygenation and enhancing hypoxemia, both in animal models (19, 20, 50) and in humans (3, 13, 30). The mechanism of this loss of contractility in response to environmental and pharmacological stimuli is not yet fully understood. How inflammation associated with infection affects pulmonary vascular responses is important, because understanding this phenomenon could at least partially explain the abnormal gas-exchange characteristic of patients with pneumonia.

Various types of K+ channels contribute to vessel tone in many vascular beds (10, 25, 37). Opening of K+ channels causes hyperpolarization of the smooth muscle cell membrane. This hyperpolarization closes L-type and T-type Ca2+ channels and reduces Ca2+ entry into the cell, resulting in lower levels of cytosolic free Ca2+ and reduced vascular tone (38, 43). NO activates large-conductance Ca2+-activated K+ (BKCa) channels, resulting in hyperpolarization of vascular smooth muscle cells, thus attenuating contractility (35, 48). In addition, although no known receptors have yet been identified, EETs and 20-HETE modulate the function of Ca2+–activated K+ (KCa) channels in vascular smooth muscle cells, thus influencing vascular contractility (24, 48). Therefore, we investigated the role of K+ channels in the attenuated pulmonary artery (PA) con-

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tractility characteristic of acute *Pseudomonas* pneumonia.

More than one type of $K^+$ channels may be involved in the relaxation of vascular smooth muscle, and this is dependent on the species and vascular bed studied (5, 6, 54). Nevertheless, the physiological role of ATP-sensitive $K^+$ ($K_{ATP}$) channels in regulating membrane potential in PAs is not certain. Thus the opening of even a few $K_{ATP}$ channels by various endogenous vasodilators and metabolic inhibitors would have substantial effects on membrane potential and vascular tone (14, 37, 39). Furthermore, levocromakalim (which opens $K_{ATP}$ channels, Ref. 8) attenuates the contractile response of small rat PAs to hypoxia, KCl, and PGF$_{2\alpha}$ (60). Glybenclamide closes $K_{ATP}$ channels in vascular smooth muscle (11). Administration of glybenclamide to animals with endotoxin-induced hypotension improves blood pressure (29, 52). This has led investigators to suggest that activation of $K_{ATP}$ channels contributes to the loss of vascular tone associated with sepsis.

Voltage-gated, delayed rectifier $K^+$ ($K_V$) current that is sensitive to blockade with 4-aminopyridine (4-AP) has been identified in vascular smooth muscle tissues (12). Specifically, a number of investigators have demonstrated a critical role for $K_V$ channels in setting the resting membrane potential in PA smooth muscle cells (2, 42, 58). In addition, inhibition of $K_V$ channels and increase in intracellular Ca$^{2+}$ have been demonstrated to play a role in the initiation of hypoxic pulmonary vasoconstriction (42, 59).

$BK_{Ca}$, $K_{ATP}$, and $K_V$ channels have all been identified in arterial smooth muscle cells (39, 54). Nevertheless, their role in regulation of vascular tone in disease has not been established. Therefore, we tested the hypothesis that $K^+$ channels contribute to the depressed PA contractility observed in the acute pneumonia model of the rat. We provide evidence for the selective involvement of $K_V$ channels in this phenomenon, suggesting new therapeutic approaches for treating the attenuated PA contractility associated with pneumonia.

**METHODS**

**Acute Pneumonia Model**

A bacterial culture (*Pseudomonas aeruginosa*) was prepared fresh weekly and was suspended in phosphate-buffered saline solution. Male Sprague-Dawley rats (330–350 g) were randomized to the pneumonia group or control group. All rats had a jugular venous line placed for fluid administration. Rats were anesthetized with halothane (1–2%) and a balance of pure oxygen. A catheter was tunneled subcutaneously from the intracapsular region to a midline incision in the neck. The Silastic venous catheter (0.023 in. ID, Clay Adams) attached to PE-50 (Clay Adams) was advanced into the superior vena cava via the right external jugular vein. The catheter was attached to a harness-swivel device (Harvard Instruments, St. Laurent, PQ) to protect the catheter and allow the rats to move freely about the cage. The incision was closed with 2-0 silk.

Animals in the pneumonia group were injected intratracheally with 0.15 ml of saline containing $3 \times 10^7$ colony-forming units/ml. A small midline incision was made to expose the trachea. A catheter was advanced through a tracheostomy into a distal bronchus for injection of the solution containing bacteria. Within 36 h, this instillation of bacteria produced an acute localized pneumonia in the left lung, with the remainder of the lung appearing grossly normal. Animals in the control group had a tracheostomy but did not have an intratracheal injection, as clinical evidence in patients has demonstrated that a febrile response with a possibility of an infection may result after bronchoalveolar lavage (44).

Postoperatively, the rats were housed separately and allowed free access to standard rat chow and water. Fluid was administered by a continuous infusion of heparinized saline (1 U/ml) at 2–3 ml/h. Fentanyl (1.0 $\mu$g/ml) was added to the intravenous infusion for analgesia.

Forty-four hours after surgery, rats were anesthetized with pentobarbital (20 mg iv), and the thorax was opened. The heart and lungs were removed en bloc, perfused through the PA with modified Krebs solution, and placed in Krebs buffer at 4°C (vide infra). Small intrapulmonary arteries were dissected out from the affected lobe in the pneumonia lung, or the corresponding lobe of lung from control rats, and studied as described below. All animals used in this study were cared for following the principles and guidelines of the Canadian Council on Animal Care and were supervised by a veterinarian. In addition, the ethics review committee at the University of Western Ontario (London, ON) approved all protocols.

**In Vitro Vascular Reactivity**

From each lung, small intrapulmonary arteries (200–400 $\mu$m diameter) were dissected out under a light microscope (Nikon Canada, Mississauga, Ontario) and were cut into 1.0- to 2.0-mm cylindrical segments. Each vessel segment was suspended on two stainless steel wires in 5-ml organ baths as described earlier (55). The two stainless steel wires were inserted through the lumen: one connected to a micromanipulator (Marzhauser MM33, Fine Science Tools, North Vancouver, BC) and the other to an FT03 force displacement transducer (Grass Instruments, Quincy, MA). Isometric tension recordings were obtained on a Grass Instruments polygraph (model 79D). The microvascular segment preparations were suspended for 30 min under an initial tension of 50 mg in Krebs solution, and placed in Krebs bicarbonate buffer solution of the following composition:

- $120 m$M NaCl
- $4.7 m$M KCl
- $1.2 m$M CaCl$_2$
- $1.2 m$M MgSO$_4$
- $1.2 m$M KH$_2$PO$_4$
- $25 m$M NaHCO$_3$
- $2.5 m$M glucose
- $7 m$M Na$_2$HPO$_4$

The heart and lungs were removed en bloc, perfused through the PA with modified Krebs solution, and placed in Krebs buffer at 4°C (vide infra). Small intrapulmonary arteries were dissected out from the affected lobe in the pneumonia lung, or the corresponding lobe of lung from control rats, and studied as described below. All animals used in this study were cared for following the principles and guidelines of the Canadian Council on Animal Care and were supervised by a veterinarian. In addition, the ethics review committee at the University of Western Ontario (London, ON) approved all protocols.

In all experiments, the preparations were equilibrated for 1 h before being used in the experiments. The vessels were then equilibrated for 1 h under a submaximal concentration of PE (120 mg/mm). The effective lumen radius (ELR) was calculated from the optimal resting wall tension for the vessels were determined in pilot experiments, as previously described, in our laboratory (49). Briefly, vessel radius was incremented from the resting ELR, and, after each stepwise increase in ELR, the baseline arterial tension and the contraction to KCl (40 mM) were recorded, followed by repeated washing with Krebs solution, until the contraction to KCl was maximal. The increase in ELR was then plotted against change in wall tension (mg/mm), and the optimal resting wall tension was determined. In all studies, vessels were tested for the presence of a functional endothelium by precontracting with a submaximal concentration of PE (2.4 $\mu$M) and relaxing with acetylcholine (10 $\mu$M) at the beginning of each experiment. All vessels that were used in this study relaxed >50% to acetylcholine.
tion (in mM): 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄·7H₂O, 1.2 KH₂PO₄, 11.1 dextrose, and 22.1 NaHCO₃ (pH = 7.4). Organ baths were continuously gassed with 95% O₂-5% CO₂.

Determination of glybenclamide concentration required to inhibit levcromakalim (KₐTP opener) effect. The concentration of glybenclamide required to inhibit levcromakalim-induced relaxation was determined. Small PAs dissected from the left lungs of naive rats (n = 8) were used. Vessels were equilibrated with vehicle (5 mg/ml sodium hydroxide and 5% glucose) or glybenclamide (10, 20, 40, 80, 160, or 320 μM) for 30 min. Arteries were precontracted with PE (2.4 μM), and a concentration-relaxation curve was constructed for levcromakalim.

Assessment of Reactivity

Contractility of intrapulmonary arteries from pneumonia and control rats was studied by using three different agonists: KCl, PE, and PGF₂α. The order of application of the three agonists to each arterial ring was randomized. With each contractile agonist, arteries were allowed to contract until a plateau was obtained before the next incremental concentration was added. After maximal contraction with each agonist was obtained, the bath was washed three to four times with fresh Krebs solution. When the vessel segments returned to the initial resting tension (~15–20 min later), the next cumulative concentration-response curve was obtained.

Role of K⁺ Channel Blockers

In arterial rings from control and pneumonia rats, log (concentration)-contraction curves were obtained to the contractile agonists KCl, PE, and PGF₂α, before and after treatment with one of the K⁺ channel blockers [10 μM paxilline or its inactive control, 10 μM paxillinol, 2 mM tetraethylammonium (TEA), 80 μM glybenclamide, 2 mM 4-AP] or their vehicle controls (DMSO for paxilline and paxillinol, and 5 mg/ml sodium hydroxide and 5% glucose for glybenclamide). Individual rings were used for each blocker or vehicle.

In arteries treated with glybenclamide (80 μM) or vehicle, the log (concentration)-relaxation curves to levcromakalim (a selective KₐTP channel opener) were also determined.

Reagents

1-PE hydrochloride, acetylcholine iodide, glybenclamide, TEA, and 4-AP were purchased from Sigma Chemical (St. Louis, MO). Levcromakalim (BRL-38227) was a gift from SmithKline Beecham pharmaceuticals. KCl (BDH Chemicals) solution (2.0 M) was freshly prepared in distilled water when needed. PGF₂α (dinoprostone tromethamine, Upjohn) was purchased from the hospital pharmacy. Stock solutions of PE (10 mM), PGF₂α (10 mM), acetylcholine (10 mM), and levcromakalim (10 mM) were prepared in distilled water and frozen (~4°C) in aliquots until needed. Glybenclamide (10 mM) was dissolved in 25 mg/ml sodium hydroxide, rapidly diluted to 5 mg/ml with 5% glucose solution, and frozen in aliquots until needed. Paxilline and its negative control paxillinol were purchased from BIOMOL Research Laboratories, (Plymouth Meeting, PA) and dissolved in DMSO. TEA (1.0 M) and 4-AP (1.0 M) were freshly prepared in distilled water when needed.

Data Analysis

Figures 1–5 were plotted using Prism (GraphPad Software, San Diego, CA) and analyzed using the statistics package for Social Sciences (SPSS-PC, version 6.0; SPSS, Chicago, IL) and GraphPad Instat (GraphPad Software). Each log (concentration)-response curve was plotted using non-linear regression analysis for dose-response curves, and the EC₅₀ of agonist (where applicable) and maximal developed wall tension (Tmin) values were determined for comparison. To study the effect of pneumonia on responses of PAs to different contractile agonists and to determine whether the K⁺ channel blockers altered these responses, cumulative concentration-contraction curves were compared between pneumonia and control groups by using repeated-measures ANOVA. The rest of the data were analyzed by using other statistical tests, as reported in RESULTS. Results are expressed as means ± SE of n values, where n is the number of rats. A value of P < 0.05 was considered significant.

RESULTS

Role of BKCa Channels

Log (concentration)-contraction curves were obtained for the contractile agonists KCl, PE, and PGF₂α on rings before and after treatment with paxilline or with its negative control, paxillinol (both at 10 μM with 10-min equilibration in organ bath). Paxilline (10 μM) has been previously shown to act as a selective and reversible blocker of large-conductance Ca²⁺-activated channels, whereas the structural analog paxillinol had no effect on these channels (28). Individual PA rings, from control and pneumonia rats, were used for paxilline or paxillinol. DMSO was used as vehicle control for paxilline and paxillinol with a final organ bath concentration of 0.001%, which did not alter baseline tone or contractile responses of arterial rings. Similarly, log (concentration)-contraction curves were obtained to the contractile agonists KCl, PE, and PGF₂α on rings before and after treatment with TEA (2 mM; 10-min equilibration in organ bath), a blocker of large-conductance Ca²⁺-activated channels (37). TEA (2 mM) did not modify baseline tone of arterial rings. Arteries from pneumonia animals had significantly depressed contractile responses to KCl, PE, and PGF₂α compared with control (Fig. 1). Neither of the BKCa blockers (paxilline and TEA) had any significant effect on the depressed contractility observed in PA rings from pneumonia rats, because the log (concentration)-contraction curves to KCl, PE, and PGF₂α, obtained before and after incubation with these blockers were not significantly different (Fig. 1). In addition, contractile responses obtained in the presence of paxilline (the inactive structural control for paxilline) were similar to those obtained with paxilline in Fig. 1. Therefore, paxilline and TEA, at concentrations known to block BKCa channels, could not reverse the attenuated PA contractility in pneumonia. This indicated that BKCa channels were not involved in this phenomenon and led us to investigate the role of other K⁺ channels.

Role of KₐTP Channels

Preliminary experiments were performed to establish the concentration of glybenclamide required to fully block KₐTP channels in arterial rings in our experimental setup. To accomplish this, the concentration of glybenclamide required to inhibit levcromakalim-in-
ROLE OF POTASSIUM CHANNELS IN ACUTE PNEUMONIA

PA rings from pneumonia rats. Values are means had no significant effect on the depressed contractility observed in facilities. Shown are log (concentration)-contraction curves to KCl (not alter the effect of pneumonia on pulmonary artery (PA) contractions in B phenylephrine (PE; was constructed for the K ATP channel opener levcromakalim (2.4 mg/mm, n = 9) and control (n = 9) rats before and after treatment with paxilline (10 μM, n = 6) or tetraethylammonium (TEA; 2 mM, n = 3). Neither paxilline nor TEA had any effect on contractility of PA rings from control rats (data not shown). In addition, Both BKCa blockers had no significant effect on the depressed contractility observed in PA rings from pneumonia rats. Values are means ± SE of observations in n rats. *Significant difference for the effect of pneumonia (or pneumonia + paxilline or pneumonia + TEA) compared with control, P < 0.05.

Reduced relaxation was determined in arteries from naive rats (Fig. 2). Arteries were precontracted with PE (2.4 μM), and a log (concentration)-relaxation curve was constructed for the KATP channel opener levcromakalim (see representative traces in Fig. 2A). A range of doses of glybenclamide (10–320 μM) was studied, as described in METHODS. Glybenclamide at the lower concentrations tested (10, 20, and 40 μM) blocked the levcromakalim relaxant response of the PE-contracted PA rings but not when higher concentrations of levcromakalim (>100 μM) were achieved in the organ bath. Glybenclamide at 80 μM fully blocked the relaxant responses to levcromakalim without significantly affecting the PE response of these arteries (control PE preconstriction = 83.3 ± 11.5 mg/mm, n = 8, compared with PE preconstriction in presence of 80 μM glybenclamide = 63.3 ± 11.3 mg/mm, n = 5, not significant). Glybenclamide at 160 and 320 μM also fully inhibited the levcromakalim relaxant response. However, these higher concentrations of glybenclamide also significantly attenuated the PE precontractile responses (28.6 ± 8.4 mg/mm, n = 3, and 32.7 ± 10.2 mg/mm, n = 4, respectively; ANOVA followed by Tukey’s test, P < 0.05 compared with control). In the presence of higher concentrations of glybenclamide, it appeared as though levcromakalim caused a contraction of naive arteries. However, what really occurred was a restoration of the PE contraction in arterial rings treated simultaneously with levcromakalim and high concentrations of glybenclamide. Note that this response was variable and only significant for the 160 and 320 μM concentrations of glybenclamide and 10–100 μM levcromakalim. Thus glybenclamide at 80 μM was the lowest dose found to fully block the relaxant responses to levcromakalim in rat PAs without having significant effects on contractility of these arteries to PE (Fig. 2B).

To assess the role of KATP channels in pneumonia, arterial rings from pneumonia or control rats were equilibrated with vehicle (5 mg/ml sodium hydroxide and 5% glucose) or glybenclamide (80 μM) for 30 min.
before assessment of contractility to KCl, PE, and PGF\textsubscript{2\alpha} (Fig. 3). In addition, log (concentration)-relaxation curves were constructed to levocromakalim in these arterial rings (Fig. 3) to confirm blockade of K\textsubscript{ATP} channels by glybenclamide at 80 \textmu M, the dose of K\textsubscript{ATP} blocker determined earlier to be effective in PA rings from naive rats. Neither vehicle nor glybenclamide had any effect on baseline tone in any of the PA rings studied. The K\textsubscript{ATP} blocker glybenclamide had no significant effect on the depressed contractility observed in PA rings from pneumonia rats (Fig. 3). Therefore, glybenclamide at a concentration (80 \textmu M) that fully blocked K\textsubscript{ATP} channels in pulmonary vessels from control and pneumonia rats (Fig. 3D) had no effect on the depressed contractility of PA rings from pneumonia rats. This indicated that K\textsubscript{ATP} channels were not involved in this phenomenon. Therefore, the role of K\textsubscript{V} channels was next evaluated.

### Role of Kv Channels

4-AP is a blocker of K\textsubscript{V} channels (delayed rectifier K\textsuperscript{+} channel) (12). On arterial rings from pneumonia and control rats, log (concentration)-contraction curves were obtained to the contractile agonists KCl, PE, and PGF\textsubscript{2\alpha} before and after treatment with 4-AP (2 mM, 10-min equilibration in organ bath). Similar to the other K\textsuperscript{+} blockers, 4-AP (2 mM) did not modify baseline tone of arterial rings. Representative tracings of the effect of 4-AP on PE and KCl responses in PA rings from pneumonia rats are shown in Fig. 4. Note that, in PA rings from pneumonia rats, treatment with 4-AP resulted in a significant increase in contractility to cumulatively added KCl (Fig. 4A) and PE (Fig. 4B). In rings from pneumonia rats, 4-AP enhanced the contractile responses to KCl, PE, and PGF\textsubscript{2\alpha}, but this enhancement varied with the agonist tested. In PA rings from pneumonia rats, 4-AP restored the KCl contractile response to that obtained in PA rings from control rats (overlapping curves for control and pneumonia + 4-AP) but only partially restored the PE and PGF\textsubscript{2\alpha} responses (Fig. 5). In PA rings from pneumonia rats, 4-AP restored the KCl T\textsubscript{max} response to that in control arteries (Table 1, KCl). In contrast, 4-AP significantly enhanced the PE T\textsubscript{max} response but did not fully restore contractility to PE to the control level. The EC\textsubscript{50} for PE in PA rings from pneumonia rats was still significantly different from that in PA rings from control rats (Table 1, PE). 4-AP significantly reduced the EC\textsubscript{50} for PGF\textsubscript{2\alpha} but did not significantly alter the T\textsubscript{max} in rings from pneumonia compared with control rats (Table 1, PGF\textsubscript{2\alpha}). Note that 4-AP did not have any significant effect on contractility of PA rings from control rats, as shown in Fig. 5. On arterial rings from control rats, log (concentration)-contraction curves to the contractile agonists KCl, PE, and PGF\textsubscript{2\alpha} before and after treatment with 4-AP were overlapping (Fig. 5, control compared with control + 4-AP for each agonist).

None of the K\textsuperscript{+} channel blockers (paxilline, TEA, and glybenclamide) or their vehicle controls had any signifi-
significant effect on contractility of PA rings from control rats (data not shown).

DISCUSSION

The results of the present study demonstrate that BKCa channels do not contribute to the depressed pulmonary vascular contractility observed in this model of acute *Pseudomonas* pneumonia. In addition, this study shows that, although the vasodilator KATP channels are present in small intrapulmonary arteries of the rat, these channels are not involved in this attenuated contractility. In contrast, 4-AP enhances contraction in PA rings from pneumonia lungs, consistent with involvement of a Kv channel in the depressed PA contractility observed in this acute pneumonia model of the rat.

BKCa channels have been described in arteries of different vascular beds, including the pulmonary circulation (4, 34, 39, 41). In addition, slight changes in membrane potential of vascular smooth muscle have been associated with significant changes in vascular tone (26, 38, 39). Paxilline, an indole diterpene alkaloid, is a potent and selective blocker of BKCa channels (28). In addition, a structurally different blocker, TEA, is selective for BKCa channels at low concentrations (15). In our study, neither paxilline nor TEA had a Fig. 5. Voltage-gated K+ channel blocker reverses effect of pneumonia on PA contractility. Shown are log (concentration)-contraction curves to KCl (A), PE (B), and PGF2α (C) of PAs from control (n = 5) and pneumonia (n = 8) rats before and after treatment with 4-AP (2 mM). 4-AP did not have any effect on contractility of PA rings from control rats (control and control + 4-AP curves overlap). In rings from pneumonia rats, 4-AP restored the KCl response and significantly enhanced the contractile responses to PE and PGF2α (*P < 0.05). Values are means ± SE of observations in n rats.

Table 1. Effect of 4-AP (2 mM) on T_max and EC_{50} values for KCl, PE, and PGF_{2α} comparing responses of small pulmonary artery rings from control and pneumonia rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pneumonia</th>
<th>Pneumonia + 4-AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl T_{max}, mg/mm</td>
<td>92.8 ± 7.8(3)</td>
<td>47.9 ± 17.0(4)</td>
<td>86.9 ± 11.6(4)</td>
</tr>
<tr>
<td>EC_{50}, mM</td>
<td>18.0 ± 2.4</td>
<td>22.0 ± 0.8</td>
<td>17.0 ± 2.7</td>
</tr>
<tr>
<td>PE T_{max}, mg/mm</td>
<td>72.5 ± 7.8(5)</td>
<td>17.0 ± 6.0(6)</td>
<td>47.6 ± 7.8(6)</td>
</tr>
<tr>
<td>EC_{50}, mM</td>
<td>33.7 ± 4.5</td>
<td>NA</td>
<td>201.0 ± 31.0</td>
</tr>
<tr>
<td>PGF_{2α} T_{max}, mg/mm</td>
<td>139.9 ± 16.8(5)</td>
<td>54.7 ± 9.1(7)</td>
<td>82.0 ± 14.1(7)</td>
</tr>
<tr>
<td>EC_{50}, µM</td>
<td>1.0 ± 0.2</td>
<td>8.0 ± 0.2</td>
<td>3.0 ± 0.4</td>
</tr>
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</table>

Values are means ± SE; nos. in parentheses, no. of rats for T_{max} and EC_{50} for each data set. 4-AP, 4-aminopyridine; T_{max}, maximal developed wall tension; PE, phenylephrine; NA, not applicable, EC_{50} could not be calculated for these curves. Significant difference * compared with control (1-way ANOVA followed by Tukey’s multiple-comparison test); † compared with pneumonia (1-way ANOVA followed by Tukey’s multiple-comparison test): P < 0.05.
significant effect on the depressed contractility observed in pneumonia, confirming that \( \text{BK}_{\text{Ca}} \) channels are not involved in this phenomenon.

Cromakalim, levcromakalim, and other \( K^+ \) channel openers repolarize or hyperpolarize and relax vascular smooth muscle primarily via activation of \( \text{K}_{\text{ATP}} \) channels (38, 43, 47). These channels regulate the resting potential of PA smooth muscle cells and are closed by the \( \text{K}_{\text{ATP}} \) closer glybenclamide (10, 11, 43). Depending on the tissue or vascular bed studied, investigators have used low doses (10–30 \( \mu \)M) of glybenclamide to achieve blockade of \( \text{K}_{\text{ATP}} \) channels (11, 39, 40). In our study, lower concentrations of glybenclamide were not effective. Levromakalim relaxed PE-contracted PA rings from control and pneumonia rats, an effect fully blocked by 80 \( \mu \)M glybenclamide. These data demonstrate that small intrapulmonary artery rings from rats have functional \( \text{K}_{\text{ATP}} \) channels. Pretreatment with glybenclamide at 160 and 320 \( \mu \)M caused depressed contractility of the PA rings to PE. The reason for this effect of high concentrations of glybenclamide is not clear but could reflect the ability of glybenclamide at high concentrations to act as a partial agonist of \( \text{K}_{\text{ATP}} \) channels, as suggested by Cai et al. (7).

The involvement of \( \text{K}_{\text{ATP}} \) channels in altered vascular tone has been suggested. Landry and Oliver (29) showed that \( \text{K}_{\text{ATP}} \) channels, at least in part, mediate the vasodilation and hypotension observed in endotoxemic dogs (29). In support of this, Vanelli et al. (52) reported that glybenclamide restored the hypotension caused by endotoxin infusion into pigs. In our rat model, as our laboratory has previously reported (55), acute \( Pseudomonas \) pneumonia resulted in depressed PA contractility to KCl, PE, and PGF\(_{2\alpha}\). In this paper, we have confirmed that, although small intrapulmonary arteries from animals with pneumonia have functional \( \text{K}_{\text{ATP}} \) channels, pretreatment of PA rings from pneumonia rats with glybenclamide (80 \( \mu \)M) did not affect the depressed vascular contractility to KCl, PE, and PGF\(_{2\alpha}\).

\( \text{K}_{\text{ATP}} \) and \( \text{BK}_{\text{Ca}} \) channels have been implicated in the depressed contractility of systemic vessels to different agonists in endotoxin animal models (22, 46). Our data suggest that these channels are not important in the attenuated pulmonary vascular contractility in this rat model of acute pneumonia. Possible reasons for these differences include the type of insult, such as lipopolysaccharide-induced vascular hyporeactivity (9, 22, 46), compared with the depressed contractility that we observed in the inflammation associated with acute pneumonia. In addition, we cannot rule out species-dependent variations (4, 23, 52) and differences in the regulation of vascular tone among different vascular beds (6, 25, 48). Indeed, different distributions of \( \text{K}_{\text{Ca}} \) channels between central and peripheral arteries at either the endothelial or smooth muscle levels or both have been suggested (5).

\( \text{K}_{\text{V}} \) currents have been described in human PA myocytes (27). In addition, Archer et al. (2) described electrophysiologically distinct myocytes isolated from conduit and resistance rat PAs, indicating differential distribution of \( \text{K}_{\text{Ca}} \) and \( \text{K}_{\text{V}} \) channels along the vascular tree. This might explain why \( \text{K}_{\text{V}} \) channels, but not other \( K^+ \) channels, seem to play a role in the depressed contractility observed in pneumonia. In isolated, freshly dispersed smooth muscle cells from rabbit portal vein and coronary arteries, the activity of \( \text{K}_{\text{V}} \) channels is enhanced by signal transduction mechanisms involving vasoactive agonists that activate cAMP-dependent protein kinase A or protein kinase C (1, 12). Further studies are needed to determine whether these signaling pathways play a role in our pneumonia model.

In this study, we showed that, in rings from pneumonia rats, 4-AP (a selective \( \text{K}_{\text{V}} \) blocker) reversed the attenuated contractile responses to KCl, PE, and PGF\(_{2\alpha}\), indicating a role for the \( \text{K}_{\text{V}} \) channel in the depressed PA contractility in acute pneumonia. 4-AP fully restored the contractile response to KCl, whereas it only partially restored the PE and PGF\(_{2\alpha}\) responses, indicating the involvement of other factors or mechanisms in this depressed contractility. Because PE and PGF\(_{2\alpha}\) are receptor-dependent agonists, variation in restoration of contractility to these agents with 4-AP treatment might reflect differences in agonist-receptor interactions and signaling pathways between arteries from control and pneumonia rats. In addition, our laboratory recently demonstrated an attenuation in CYP metabolic activity of AA in lung microsomes from rats with pneumonia (57). In pneumonia, there is a reduction in the rate of formation of pulmonary EETs and 20-HETE, which are potent constrictors of PA rings in vitro (57) and which have been suggested to act as intracellular signaling molecules in vascular smooth muscle cells and endothelium (24, 32). Our laboratory also demonstrated depressed contractility to the CYP metabolites of AA, EETs, and 20-HETE in PA rings from rats with pneumonia. NO mediates part of the observed depressed PA contractility in pneumonia, although the mechanisms underlying this phenomenon are not fully known (55, 57). In many vascular beds, NO can activate \( \text{K}_{\text{Ca}} \) channels, resulting in hyperpolarization of vascular smooth muscle cells, thus affecting its contractility (16, 33). Although no known receptors have yet been identified, both EETs and 20-HETE could exert their contractile effects by modulating the function of \( K^+ \) and \( \text{Ca}^{2+} \) channels in vascular smooth muscle cells and modulating the effect of NO on these channels (21, 24, 36, 48). It remains to be established whether a link exists among NO, EETs, 20-HETE, and \( \text{K}_{\text{V}} \) channels in PAs of the rat.

In conclusion, our data reveal that \( \text{K}_{\text{V}} \) channels, but not \( \text{BK}_{\text{Ca}} \) and \( \text{K}_{\text{ATP}} \) channels, contribute to the depressed PA contractility observed in this model of acute \( Pseudomonas \) pneumonia. This observation is important, because it might shed the light on better therapeutic strategies for the treatment of pneumonia.

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