Pergolide Is an Inhibitor of Voltage-Gated Potassium Channels, Including Kv1.5, and Causes Pulmonary Vasoconstriction

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Background—Pergolide produces clinical benefit in Parkinson disease by stimulating dopamine D1 and D2 receptors. An increased incidence of carcinoid-like heart valve disease (CLHVD) has been noted in pergolide users, reminiscent of that induced by certain anorexigens used for weight reduction. Anorexigens that modulate serotonin release and reuptake, such as dexfenfluramine, were withdrawn from sale because of CLHVD. Interestingly, the anorexigens also caused pulmonary arterial hypertension (PAH). Anorexigens were shown to enhance hypoxic pulmonary vasoconstriction, in part by inhibiting voltage-gated K\(^+\) channels (Kv) in pulmonary artery smooth muscle cells (PASMCs). Although PAH has not been associated with pergolide use, we hypothesized that pergolide might have similar effects on hypoxic pulmonary vasoconstriction and Kv channels.

Methods and Results—Pergolide enhanced hypoxic pulmonary vasoconstriction in the isolated perfused rat lung compared with control lungs (mean pulmonary artery pressure 32±3 versus 21±2 mm Hg; \(P<0.01\)). Pergolide also caused vasoconstriction in rat pulmonary artery rings. Pergolide inhibited PASMC potassium current density, resulting in membrane depolarization (from 51±2 to 44±1 mV) and increased cytosolic calcium in both rat and human PASMCs. Pergolide directly inhibited heterologously expressed Kv1.5 and \(K_{Ca}\) channels.

Conclusions—Pergolide causes Kv channel inhibition and, despite being from a different class of drugs, has pulmonary vascular effects reminiscent of dexfenfluramine. Coupled with their shared proclivity to induce CLHVD, these findings suggest that clinical monitoring for pergolide-induced PAH should be considered. (Circulation. 2005;112:1494-1499.)

Key Words: drugs ■ hypotension, pulmonary ■ ion channels ■ muscle, smooth ■ vasoconstriction

Pergolide is an ergot alkaloid–derived medication designed to alleviate the effects of Parkinson disease by stimulating the dopamine D1 and D2 receptors.\(^1\) Although the drug has been helpful clinically, there have recently been reports of associated carcinoid-like heart valve disease (CLHVD), as well as pulmonary and retroperitoneal fibrosis.\(^2,3\) The valvulopathy associated with the use of pergolide is similar to that seen with the ergot alkaloid drugs and the anorectic agents fenfluramine and aminorex.\(^4\) Fenfluramine and aminorex are also known to be associated with an increased incidence of pulmonary hypertension.\(^5\) Despite the observation that pergolide may cause CLHVD, no clinically significant increase in the incidence of pulmonary hypertension has been recognized. We have shown previously that dexfenfluramine, fenfluramine, and aminorex inhibit potassium current (\(I_K\)) in PASMCs, leading to membrane depolarization, calcium entry, and vasoconstriction.\(^6\) Given that pergolide and fenfluramine both cause CLHVD, we wished to determine whether pergolide might also be a K\(^+\) channel blocker and cause pulmonary artery (PA) vasoconstriction, in which case it might pose a risk of pulmonary arterial hypertension (PAH). The present study shows that pergolide is an inhibitor of voltage-gated K\(^+\) channels (Kv) and causes pulmonary vasoconstriction by a mechanism similar to that initiated by dexfenfluramine.

Methods

All studies were approved by the Institutional Animal Care and Use Committee of the Minneapolis Veterans Affairs Medical Center and conform to current National Institutes of Health and American Physiological Society guidelines for the use and care of laboratory animals.

Isolated Perfused Rat Lung

Lungs were isolated en bloc from adult male Sprague-Dawley rats (weight, 250 to 350 g) after the rats were anesthetized with pentobarbital (50 mg/kg body wt IP), as previously described.\(^7\) Lungs were then perfused at a constant flow so that changes in pressure reflect changes in pulmonary vascular resistance. The perfusate was Krebs’ salt solution, balanced for osmotic potential...
with Ficoll to reduce the possible effects of pergolide binding to protein, such as albumin. Lungs were ventilated with humidified gases containing 21% O₂/5% CO₂/balance N₂ (normoxia) or 2.5% O₂/5% CO₂/balance N₂ (hypoxia). The lung chamber and perfusate were maintained at 37°C. Respiration was set to physiological values (frequency 70 breaths per minute, tidal volume 1.5 mL), with a positive end-expiratory pressure of 2.5 cm H₂O. To determine lung reactivity, we subjected the lungs to 10 minutes of normoxia and a 6-minute hypoxic challenge, without the use of angiotensin II or other vasoconstrictors. After return to baseline, the lungs were given a nitric oxide synthase inhibitor (N-nitro-L-arginine methyl ester, 50 µmol/L) and perfused for 20 minutes before 2 additional hypoxic challenges. Eight lungs then received pergolide 10 µmol/L, and 8 received the vehicle before a final hypoxic challenge.

Large Resistance PAs
Male Sprague-Dawley rats were anesthetized with pentobarbital (50 mg/kg IP). Large resistance PAs (third and fourth division, internal diameter 400 to 800 µm) were dissected and placed immediately in ice-cold Earle’s balanced salt solution of the following composition (in mmol/L): 1.8 CaCl₂, 0.8 MgSO₄, 5.4 KCl, 116.2 NaCl, 1.0 NaH₂PO₄, 5.6 glucose, and 26.2 NaHCO₃. The vessels were cleaned of connective tissue, and 4-mm-long rings were suspended in stirrups in organ chambers (Radnoti) for measurement of isometric force, as previously described. For some experiments the endothelium was mechanically denuded with the removal of endothelium, demonstrated by the absence of the normal relaxation response to acetylcholine (0.1 to 10 µmol/L). Chambers were filled with Earle’s balanced salt solution and bubbled with 21% O₂/5% CO₂/balance N₂ (normoxic) and 2.5% O₂/5% CO₂/balance N₂ (hypoxic) gases. Temperature was kept at 37°C, and pH was 7.35 to 7.45. The rings were equilibrated at a resting tension of 900 mg for at least 60 minutes and then exposed 3 times to phenylephrine (1 µmol/L) at 30-minute intervals. The optimal resting tension was that which permitted the maximal constriction to 80 mmol/L KCl in preliminary experiments. After an additional 30 minutes, pergolide was given at a concentration of 10 µmol/L.

Small Resistance PAs
Small resistance PAs (internal diameter 150 to 400 µm) were dissected free of adventitia, and the rings were mounted in a temperature-controlled myograph at 37°C (Multi Myograph System-610M, Danish Myo Technology A/S). They were bubbled continuously with 21% O₂/5% CO₂ (pH 7.35 to 7.45). The rings were equilibrated to give a transmural pressure of 400 mg. After at least 60 minutes of this tone, the viability of the endothelium was assessed, as determined by relaxation in response to 1 µmol/L acetylcholine. At this point the effect of pergolide (10 µmol/L) was observed.

Cell Dispersal
PA smooth muscle cells (PASMCs) were dispersed freshly on the day of electrophysiological study. Sprague-Dawley rats were anesthetized, and the lungs were removed and perfused with Ca²⁺-free Hanks’ solution. The Hanks’ solution contained (in mmol/L) 140 NaCl, 4.2 KCl, 1.2 KH₂PO₄, 0.5 MgCl₂, 10 HEPES, and 0.1 EGTA (pH 7.4). Fourth division PAs were dissected and placed in the Hanks’ solution for 10 minutes at 4°C. The arteries were then placed in Hanks’ solution containing 1 mg/mL of papain, 0.75 mg/mL bovine albumin, and 0.85 mg/mL of diithiothreitol without EGTA and digested at 4°C for 30 minutes and then at 37°C for 13 minutes. Finally, the arteries were washed thoroughly with Hanks’ solution without EGTA (“low-Ca²⁺”) for at least 10 minutes and maintained on ice in Hanks’ solution supplemented with 1 mg/mL glucose. This digestion protocol consistently produced high yields of viable, relaxed SMCs.

Cell Electrophysiology
Gentle trituration produced a cell suspension that was divided into aliquots and placed in a perfusion chamber on the stage of an inverted microscope (Diaphot 200, Nikon) for conventional whole-cell patch-clamp studies. After a brief period to allow adherence to the bottom of the recording chamber, cells were perfused with a normoxic solution of the following composition (in mmol/L): 115 NaCl, 25 NaHCO₃, 4.5 KCl, 1 KH₂PO₄, 1.5 CaCl₂, 0.5 MgCl₂, 10 HEPES, and 10 glucose (pH 7.4 with NaOH, Po₂ 120 mm Hg by bubbling with 21% O₂/5% CO₂/balance N₂). Electrode resistances ranged from 3 to 5 MΩ after fire polishing and when filled with a solution of composition (in mmol/L) 140 KCl, 1.0 MgCl₂, 10 HEPES, 1 EGTA, 1 KATP (pH 7.2 with KOH). The patch-clamp amplifier was Axopatch 200B (Axon Instruments) in all voltage- and current-clamp experiments. Offset potentials were nulled directly before formation of a seal. Capacitance and series resistance were corrected (usually 80%). No leak subtraction was made. Leakage current was monitored with the use of hyperpolarizing steps (~30 mV) from the holding potential, a procedure that did not activate ion channels but allowed measurement of passive membrane properties and leak during the experiments. Cells expressing holding current at ~70 mV of >20 pA before or during the recordings were discarded. The extracellular perfuse perfusion rate was 2 mL/min. Cells were voltage clamped at a holding potential of ~70 mV, and currents were evoked by 20-mV steps to more positive potentials with test pulse protocol (50/0/500 ms). Currents were filtered at 1 kHz and sampled at 2 or 4 kHz. For resting membrane potential (Eₘ), cells were held in current clamp at their resting Eₘ (without current injection). Series resistance and leak were checked at the beginning and end of each Eₘ experiment to eliminate artificial changes in potential. Data were recorded and analyzed with pClamp 8 software (Axon Instruments).

CHO Cells Transfected With Kv1.5 or KCa
Kv1.5 from human PA or BKα, cloned from rat PA was placed in serotype 5 adenovirus with a green fluorescent protein reporter under a CMV promoter. CHO cells were transfected with these vectors, and whole-cell patch clamp was performed on green cells, as previously described.

Human PASMC Cell Culture
Cultured human PASMCs (fourth passage) were obtained from Clonetics (Walkersville, Md) and plated in smooth muscle growth medium (Clonetics) supplemented with gentamicin (50 µg/mL), human epidermal (0.5 µg/mL) and fibroblast growth factors (1.0 µg/mL), insulin (5 µg/mL), and 5% fetal bovine serum. Cells were grown at 37°C in a humidified atmosphere of 5% CO₂. After they reached confluence, cells were dislodged from the dish by treatment with Accutase (TCS Biologicals) and transferred to T75 flasks for further passage.

Calcium Imaging
Dual-excitation imaging with fura 2 was used to measure cytosolic Ca²⁺. Dispersed PASMCs were loaded with fura 2-AM following a standard protocol. Cells were then transferred to imaging plates (Molecular Probes) and incubated in low-Ca²⁺ Hanks’ solution. The plates were washed in the same solution and placed on the microscope stage. All drugs were given as a bolus by direct microinjection. Volumes were limited to 10 µL, and similar volumes of saline had no effect on [Ca²⁺]. Changes in [Ca²⁺] were recorded in individual cells with a MetaFluor-driven 340/380 filter imaging system (Universal Imaging) and cooled charge-coupled device camera (Photometrics).

Statistical Analysis
Data are expressed as mean±SE. The effects of drugs on Lₚ and PA pressure were compared with repeated-measures ANOVA (Statview II, version 4.0, Abacus Concepts). Membrane potential data were compared with the Student paired t test. A value of P<0.05 was considered significant.

Results
Isolated Perfused Lung
Pergolide (10 µmol/L) increased normoxic PA pressure from 10.3±1.5 to 15.6±3.7 mm Hg (P<0.01) and also increased...
hypoxic pulmonary vasoconstriction from control hypoxic pulmonary vasoconstriction of 20.8 ± 2.0 mm Hg to 32.2 ± 3.1 mm Hg (n=8 each; P<0.01) (Figure 1).

PA Rings
Pergolide (10 μmol/L) caused contraction in rings from both large- and small resistance PAs. Whereas phenylephrine-induced contraction of large proximal PA rings was 380.1 ± 27.5 mg, 10 μmol/L pergolide caused a vasoconstriction of 134.5 ± 16.7 mg, which is 38.5 ± 4.8% when expressed as a percentage of the phenylephrine contraction (n=15) (Figure 2A). The constriction caused by pergolide was inhibited by an L-type calcium channel blocker, nifedipine (1 μmol/L: 73.2 ± 7.9%, n=5; 3 μmol/L: 99.3 ± 2.3%, n=5) (Figure 2B). Pergolide 10 μmol/L also caused potent vasoconstriction in small resistance PAs, which was 54.9 ± 6.8% of phenylephrine contraction (n=8) (Figure 2B).

Electrophysiology
Pergolide (10 μmol/L) inhibited IK in resistance PASMCs from 166.3 ± 33.8 to 69.0 ± 10.5 pA/pF at 50 mV (Figure 3). The effect was rapidly reversed when the drug was washed out. Pergolide (10 μmol/L) depolarized PASMC membrane potential from −51.1 ± 1.5 to −44.0 ± 0.8 mV in resistance PASMCs (Figure 4) (n=5). In CHO cells, pergolide (10−7 to 10−5 mol/L) inhibited heterologously expressed rat BKCa channels in a dose-dependent manner (n=5) (Figure 5A). It is also clear that pergolide inhibited heterologously expressed human Kv1.5 (n=6), an important O2-sensitive Kv channel expressed in PASMCs (Figure 5B).

Calcium Imaging
Average resting [Ca2+]i in freshly isolated rat PASMCs was calculated to be 167 ± 16 nmol/L (n=12). Pergolide 10 μmol/L caused an increase in [Ca2+]i to 231 ± 18 nmol/L (P<0.001) (Figure 6). Average resting [Ca2+]i, in cultured human PASMCs was calculated to be 41 ± 6 nmol/L (n=11). Pergolide 10 μmol/L caused an increase in [Ca2+]i to 156 ± 55 nmol/L in cultured human PASMCs (P=0.06) (Figure 6).
Discussion

During the 15 years since it was introduced, >1.7 million patients with Parkinson disease have been treated with pergolide. These patients have displayed evidence of some serious side effects, including retroperitoneal and pericardial fibrosis. However, it was only in 2002 that CLHVD was recognized as a possible drug effect.\textsuperscript{10,11} Since then, an increased incidence of valvular heart disease has been described in 3 additional echocardiography studies.\textsuperscript{12–14} In one of these, there was a major suspicion of restrictive valvular disease in 19% of those taking pergolide, as compared with 0% in the control group.\textsuperscript{14}

In the case of the drug dexfenfluramine, longer use led to CLHVD and was also associated with pulmonary hypertension.\textsuperscript{5,15} PAH has not been recognized to be a complication of the use of pergolide. However, in clinical observations the estimated PA pressure was significantly higher in 15 patients treated with pergolide than in controls. The systolic PA pressure in 1 patient was estimated to be as high as 71 mm Hg.\textsuperscript{14} The cause of the observed elevated pressure is not clear. It could be the result of pulmonary fibrosis, valvular regurgitation, or several other etiologies, but PA systolic pressure was still elevated when patients with significant mitral regurgitation were excluded.\textsuperscript{14} However, in view of the present observations and our previous findings on the mechanism of dexfenfluramine-induced pulmonary hypertension,
Figure 7. Pergolide inhibits PASMC K+ channels, depolarizes cell membrane potential, and causes the calcium influx through voltage-gated calcium channels (VGCC).

it is possible that pergolide has a direct effect on the pulmonary vasculature. The concentration of pergolide (10 μmol/L) that we used in most of these studies is likely to be in the range of the plasma concentration in patients taking the drug. After a single oral dose of 0.138 mg in healthy volunteers, the peak plasma concentration was ≈4.4 μmol/L. The mean therapeutic dose of pergolide is 1 mg given 3 times per day, suggesting that the plasma concentration would be significantly higher. However, pergolide is >90% bound to plasma proteins, and therefore the free concentration is uncertain. There is also evidence of rapid uptake by tissues, raising the possibility of protracted activity.

These experiments demonstrate that pergolide causes an increase in PA pressure in isolated perfused lungs and contraction of resistance PA rings. The cellular electrophysiology suggests that the mechanism involves inhibition of IK, membrane depolarization, and Ca2+ entry through voltage-gated L-type Ca2+ channels. The specific potassium channel was further defined by examining the effect of pergolide on Kv1.5 and KCa channels transfected into CHO cells. The drug inhibited both channels. The conclusion that calcium influx is responsible for the vasoconstriction is suggested by the observation that the vasoconstriction was almost completely reversed by nifedipine. Dexfenfluramine causes pulmonary vasoconstriction by the same mechanism. However, the major metabolite of dexfenfluramine, nordexfenfluramine, causes pulmonary vasoconstriction predominantly through the release of Ca2+ from the sarcoplasmic reticulum.18,19 Given the impressive effect of nifedipine, this does not seem to play a significant role in the case of pergolide. The proposed mechanism by which pergolide may cause pulmonary vasoconstriction is shown in Figure 7. It is interesting that pergolide has been reported to block hERG K+ current and shorten the potential duration of Purkinje fibers. Another mechanism that could be invoked to explain pergolide-related valvular disease and possible pulmonary hypertension involves the serotonin 5-HT2A receptor. Pergolide activates this receptor, which has been implicated in fenfluramine-induced valvular disease and in pulmonary hypertensive arterial disease.

Another mechanism that could not explain the inhibition of IK or the entry of calcium through L-type calcium channels in the PASMCs. Is there a connection between the serotonin receptor–mediated effect and the K+ channel effect of pergolide? Activation of the bone morphogenetic protein 2 (BMP2) receptor is thought to have an inhibitory effect on serotonin signaling. Some patients with idiopathic PAH have a “loss of function” mutation of the BMP2 receptor. It has recently been reported that BMP2 increases K+ channel function/expression. Consequently, loss of BMP2 signaling may permit serotonin proliferative signals to be unopposed by K+ channel effects. Because fenfluramine and pergolide both stimulate serotonin receptors and inhibit K+ channel function/expression, it is not surprising that vasoconstriction, proliferation, and a decrease in apoptosis might follow in PASMCs.

The pathophysiology of idiopathic PAH includes vasoconstriction, cellular proliferation, and thrombosis in small vessels. This work shows that pergolide can cause significant vasoconstriction. In addition, the observed intracellular effects might also initiate cellular proliferation. Pergolide may cause vascular remodeling through 2 separate mechanisms. Increased intracellular potassium, induced by the inhibition of potassium channels, has been shown to be antiapoptotic and increased intracellular calcium has been shown to promote cellular proliferation. Further evaluation of the clinical safety of pergolide is indicated, with special attention to the possibility that PAH might occur in chronic users.

Acknowledgments

Dr Weir is supported by Veterans Affairs Merit Review Funding and National Institutes of Health grant RO1-HL65322. Dr Archer is supported by the Canadian Institutes for Health Research, Canada Foundation for Innovation, National Institutes of Health grant RO1-HL071115, the Canadian Institutes for Health Research (CIHR), and ABACUS, the Alberta Cardiovascular and Stroke Research Centre. Dr Archer is a Canada Research Chair in Oxygen Sensing and Translational Cardiovascular Research.

References


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**CLINICAL PERSPECTIVE**

Anorectic agents, such as fenfluramine, were associated with a carcinoid-like thickening and restriction of the heart valves, in addition to pulmonary hypertension. Recently, a dopamine receptor agonist, pergolide, used in the treatment of Parkinson disease, was described to cause a similar valvulopathy. It is important to know whether it might also have the potential to cause pulmonary hypertension. Patients with idiopathic pulmonary arterial hypertension have decreased expression/function of a specific potassium channel in the pulmonary arterial smooth muscle cells. The normal function of potassium channels helps to keep the small pulmonary arteries dilated and to inhibit cell proliferation. In this issue Hong et al report that pergolide causes inhibition of potassium channels, membrane depolarization, calcium entry, and vasoconstriction in a manner similar to that of fenfluramine. In view of these findings and the observation of elevated pulmonary artery pressures in some patients taking pergolide, attention should be paid to the possibility that pulmonary hypertension might occur in chronic users.
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_Circulation_. 2005;112:1494-1499; originally published online August 29, 2005; doi: 10.1161/CIRCULATIONAHA.105.556704

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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