Inhibition of Vascular Smooth Muscle Cell Migration by Elevation of Extracellular Potassium Concentration

Ge Ma, David P. Mason, David B. Young

Abstract—The effect of potassium on the migration of vascular smooth muscle cells was analyzed in media made with extracellular potassium concentrations of 3, 4, 5, and 6 mmol/L. The migration of cultured porcine coronary artery cells was stimulated with platelet-derived growth factor (PDGF)-BB. In the first study, cells were exposed to PDGF-BB at concentrations of 0, 10, or 20 ng/mL for 5 hours with the use of a Boyden chamber. Cells were quiescent overnight in 0.5% fetal bovine serum in Dulbecco’s modified Eagle’s medium with an extracellular potassium concentration of 4 mmol/L. With increasing potassium concentration, migration was significantly inhibited (P<0.02, 2-way ANOVA). In the cells exposed to 10 ng/mL PDGF-BB, migration ranged from 500±86% to 294±44% (value in wells with 0 ng/mL PDGF-BB and 4 mmol/L potassium concentration=100%) in medium containing 3 to 6 mmol/L extracellular potassium concentration (P<0.03). Long-term potassium exposure was investigated in cells grown in 5% serum in Dulbecco’s modified Eagle’s medium with an extracellular potassium concentration of 3, 4, 5, or 6 mmol/L for 3 to 4 weeks. Migration was assessed with 0 or 20 ng/mL PDGF-BB. Migration was significantly inhibited by the elevation of extracellular potassium concentration (P<0.01, 2-way ANOVA). With 20 ng/mL PDGF-BB, the migration rates ranged from 152±11% in medium with 3 mmol/L potassium to 69±5% in 6 mmol/L potassium (P<0.01). Increases in extracellular potassium concentration within the physiological range significantly and directly inhibit vascular smooth muscle cell migration. (Hypertension. 2000;35:948-951.)

Key Words: arteries • atherosclerosis • neointima • platelet-derived growth factor

In recent studies in this laboratory, an inverse relationship was observed between dietary potassium content and the severity of neointimal proliferation after balloon angioplasty.1–2 In experiments with rats and pigs, animals fed a high-potassium diet had significantly less neointimal area at the site of injury than animals fed a normal- or low-potassium diet. The cause of the neointimal proliferative lesion that occurs after angioplasty has been studied extensively by others and is known to include several alterations in the function of cells of the arterial wall. Of significant importance are the migration from the media to the subintima and the subsequent proliferation of medial vascular smooth muscle cells. During migration to the intima, the cells change from the differentiated, contractile phenotype to the dedifferentiated, synthetic state that is associated with proliferation.3–6 Platelet-derived growth factor (PDGF) is a potent chemoattractant and mitogen for cells of mesenchymal origin and for vascular smooth muscle cells.7–10

Previously, results from experiments in our laboratory have been consistent with the hypothesis that elevated extracellular potassium concentration inhibits the functions of platelets and vascular smooth muscle cells, including proliferation,11–12 believed to be involved in neointimal proliferative lesion formation. The purpose of the present study was to extend those results by testing the hypothesis that the elevation of extracellular potassium concentration inhibits vascular smooth muscle cell migration. An in vitro design with a modified Boyden chamber was used so that precise control of potassium concentration and other variables could be maintained.

Methods

Vascular Smooth Muscle Cell Isolation and Culture

Vascular smooth muscle cells were obtained from microdissected explants of the left circumflex coronary artery of castrated male 6- to 8-week-old common swine. Connective tissue–free pieces of medial vascular smooth muscle were placed in culture dishes with 10% fetal bovine serum (FBS) in Dulbecco’s modified Eagle’s medium (DMEM) with 100 U/mL penicillin, 100 µg/mL streptomycin, and 25 µg/mL gentamicin. The cells were passaged once and stored frozen at −80°C or in liquid nitrogen. After thawing, they were grown in 5% FBS in DMEM with 100 U/mL penicillin, 100 µg/mL streptomycin, and 25 µg/mL gentamicin before being studied. The...
selectivity of the isolation technique and the purity of the cultures were analyzed in 4 isolations with immunohistochemical staining in passage 3 for α-smooth muscle actin and for vimentin for fibroblasts.13 All of the cells were positive for actin, and no cells were positive for vimentin.

Short-Term Experiments
Cells were cultured in 5% CO₂/95% room air in 150-mm² flasks at 37°C in 5% FBS in DMEM with a potassium concentration of 4 mmol/L until 80% confluent. They were made quiescent overnight in 0.5% FBS in DMEM with a potassium concentration of 4 mmol/L and then released from the surface with trypsin. Cells were centrifuged in 10% FBS in DMEM with a potassium concentration of 4 mmol/L and diluted to 10⁶ cells/mL. The volume containing the cells was divided into 4 aliquots that were added to tubes containing 10% FBS in DMEM with potassium concentrations of 3, 4, 5, and 6 mmol/L; centrifuged; and resuspended in serum-free DMEM with potassium concentrations of 3, 4, 5, or 6 mmol/L at a cell density of 10⁶ cells/mL.

A modified 48-well Boyden chamber was used to assess the migration rate.3,14,15 The chambers were separated with a polypyrrolidone-free polycarbonate filter with 8-μm pores that had been coated with Matrigel (40234-A; Becton Dickinson). After exposure for 15 to 30 minutes to the selected potassium concentrations, 53 μL of the serum-free cell suspensions was placed in the upper chamber minute, whereas 28 μL of serum-free DMEM with the same potassium concentration as in the upper chamber and with or without PDGF-BB was placed in the lower chamber. PDGF-BB concentrations chosen to stimulate vascular smooth muscle cell migration were 10 or 20 ng/mL.3,7,14 Migration was allowed to proceed for 5 hours at 37°C in 5% CO₂. The lower side of the membrane was fixed and stained for cell counting (B4132-1; Dade AG). The migrated cells were then quantified with computerized image analysis (Optimas 5; Optimas Corp).

Long-Term Experiments
After thawing, the cells were grown in 5% FBS in DMEM with potassium concentrations of 3, 4, 5, or 6 mmol/L for 3 to 4 weeks, reaching 80% confluent in passage 4 to 5. They were made quiescent overnight in 0.5% FBS in DMEM with the selected potassium concentration, trypsinized, centrifuged in 10% FBS in DMEM, washed with serum-free DMEM, and resuspended in serum-free DMEM at a density of 10⁶ cells/mL, all in solutions with the same potassium concentrations in which they had been cultured for 3 to 4 weeks. Then, 53 μL of the cell suspensions was added to the upper wells after a 15- to 30-minute preincubation, whereas 28 μL of serum-free DMEM with the same potassium concentrations as in the upper chambers together with either 0 or 20 ng/mL PDGF-BB was used in the lower chambers. Incubation proceeded under the same conditions used in the short-term experiment, except that migration time was 5 hours 30 minutes in the long-term study.

Data Analysis
Data are presented in the text and figures as group mean±SEM values. Two-way ANOVA was used to test for significance of the effects of PDGF and potassium concentration among groups. When the results of ANOVA suggested a significant difference among groups, mean group values were tested for difference with Dunnett’s modification of the Student’s t test for independent measures. Correlation coefficients were calculated and are presented in the text with their associated probability values. A probability value of <5% was considered statistically significant.

Results
Short-Term Effect of Potassium Concentration on Migration
In the short-term study, vascular smooth muscle cells exposed to no growth factors and to a control potassium concentration of 4 mmol/L migrated through the membrane to the lower chamber, reaching a mean count of 93±10 cells in the 5-hour experiment. Data from the experiment in which the cells were exposed to PDGF-BB or to changed medium potassium concentration for 5 hours, or both, are presented in Figure 1, expressed as percentages; 100% is the mean value for migration in a potassium concentration of 4 mmol/L and 0 ng/mL PDGF-BB within each experiment. The addition of PDGF-BB to the lower chambers increased the migration rate. The stimulatory effect of PDGF-BB on migration has been shown by others to decline with increases in concentration above 10 ng/mL.3,7,14 and the same trend was observed in the present study, with the group mean of the 10 ng/mL counts being significantly greater than the 20 ng/mL group mean at each level of potassium concentration. With increasing potassium concentration, migration was significantly inhibited (P<0.02, 2-way ANOVA; for 10 ng/mL, r=-0.22, P<0.01; for 20 ng/mL, r=-0.13, P<0.09). In the wells with 10 ng/mL PDGF-BB, the migration values ranged from 500±86% for 3 mmol/L potassium to 294±44% in the wells containing 6 mmol/L potassium concentration (P<0.03).

Long-Term Effect of Potassium Concentration on Migration
The effects of long-term exposure of the cells to selected extracellular potassium concentrations were also investigated. Cells were grown in medium with concentrations of 3, 4, 5, or 6 mmol/L for 3 to 4 weeks before being added to the wells of the Boyden chamber. In the upper and lower chambers, the potassium concentrations were the same as those in which the cells had been grown. Migration was assessed with either 0 or 20 ng/mL PDGF-BB in the lower chambers. The migration data expressed as percentage of the control values are presented in Figure 2. Migration rate was significantly inhibited by elevation of potassium concentration (P<0.01, 2-way ANOVA; for 20 ng/mL, r=-0.65, P<0.01; for no PDGF-BB, r=-0.52, P<0.01). With 20 ng/mL PDGF-BB, the migration rates associated with changes in potassium concentration ranged from 152±11% in medium with a
potassium concentration of 3 mmol/L to 69±5% in 6 mmol/L potassium (P<0.01).

**Discussion**

Cell migration can be studied with several techniques. The Boyden chamber allows the investigator to accurately control conditions of the experiment such as concentrations of the agonists, the duration of exposure, and the source and stage of development of cells. Most important, the chamber design enables the investigator to obtain a quantitative measure of migration; furthermore, quantitative precision was increased in this study with computer-driven image analysis to determine cell numbers. With this sensitive technique, increases in potassium were observed to consistently reduce the rate of migration of vascular smooth muscle cells, with and without stimulation from PDGF-BB and regardless of whether the cells were exposed to the selected potassium concentration for 5 hours or for 3 to 4 weeks.

The effects of increases in potassium concentration on cell migration may be mediated via stimulation of the activity or expression of Na\(^+\),K\(^+\)-ATPase in the cell membrane, which would decrease intracellular sodium concentration and increase the concentration gradient driving the sodium–calcium exchange mechanism. In studies in vascular smooth muscle, the reduction in tension associated with an elevation of potassium concentration in the physiological range has been attributed by Haddy and Scott\(^{16–18}\) to an increase in Na\(^+\),K\(^+\)-ATPase activity. In addition, Songu-Mize et al\(^ {19}\) demonstrated that physiological increases in dietary potassium intake or in plasma potassium concentration act in vivo to increase vascular smooth muscle Na\(^+\),K\(^+\)-ATPase activity. An increase in the physiological range in extracellular potassium concentration was shown by Jones\(^ {20}\) to increase sodium efflux from vascular smooth muscle cells in vitro. Similarly, in other cell types, increased expression of Na\(^+\),K\(^+\)-ATPase in the cell membrane has been associated with a reduction in intracellular sodium concentration.\(^ {21}\) Any reduction in intracellular sodium concentration associated with an elevation of extracellular potassium concentration could be expected to stimulate calcium extrusion from the cell via the sodium–calcium exchange mechanism and subsequently lead to a reduction in intracellular calcium concentration. Because the cellular locomotion required for migration is mediated by coordinated, calcium-dependent changes in the polymerization and depolymerization of actin-containing microfilaments,\(^ {22}\) any reduction in intracellular calcium activity associated with an elevation of extracellular potassium concentration could be expected to reduce migration.

The migration of vascular smooth muscle cells into the subintima is an early and quantitatively important step in atherosclerotic and restenosis lesion formation.\(^ {23,24}\) In the rat, most of the cells of the injury lesion after angioplasty are progeny of cells that migrated from the media, whereas in the swine and human restenosis lesions, the modified smooth muscle cells that compose the lesion are derived from cells that originated in the intima as well as those from the media.\(^ {24}\) The inhibitory effect of potassium on migration together with an effect on proliferation may account for the large magnitude of reduction in neointima formation associated with a high dietary intake of potassium observed in the angioplasty studies in rats and pigs.\(^ {1,2}\)

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