Blockade of the ATP-Sensitive Potassium Channel Modulates Reactive Hyperemia in the Canine Coronary Circulation

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The mechanism of reactive hyperemia remains unknown. We hypothesized that reactive hyperemia was related to the opening of ATP-sensitive potassium channels during coronary occlusion. The resulting hyperpolarization of the smooth muscle cell plasma membrane might reduce calcium influx through voltage-dependent calcium channels and result in relaxation of smooth muscle tone and vasodilation. In eight open-chest, anesthetized dogs, 30-second coronary occlusions resulted in an average flow debt repayment of 200 ± 41%. After low-dose (0.8 μmol/min) and high-dose (3.7 μmol/min) infusion of intracoronary glibenclamide, flow debt repayment fell to 76 ± 14% and 50 ± 8%, respectively (p < 0.05 compared with control for both). The decline in flow debt repayment was due to a significant reduction both in maximum coronary conductance during reactive hyperemia and in its duration. In addition, there was a significant decline in the sensitivity of the coronary circulation to adenosine-induced vasodilation after glibenclamide. While more variable, there was no overall change in the sensitivity of the coronary vasculature to acetylcholine-induced vasodilation after glibenclamide. We conclude that reactive hyperemia is determined in a large part by the ATP-sensitive potassium channel, probably through its effect on membrane potential and voltage-sensitive calcium channels. Because reactive hyperemia was never fully abolished at the highest doses of glibenclamide tested, it is possible that additional mechanisms are involved in the genesis of this complex phenomenon. (Circulation Research 1991;69:618–622)

The mechanism underlying reactive hyperemia of the coronary circulation remains unknown. A large body of circumstantial evidence developed during the 1960s and 1970s implicated adenosine as the most likely mediator of this response because adenosine increases during brief coronary occlusions and declines over time after occlusion release in a manner that parallels changes in coronary flow and conductance. A critical study by Saito et al showed, however, that adenosine could account for no more than 30% of the reactive hyperemia of the myocardium. Since that time little additional light has been shed on the mechanism of this phenomenon.

Recently, an ATP-sensitive potassium channel has been reported to exist in vascular smooth muscle cells. Blockade of this channel in the isolated guinea pig heart with the sulfonylurea derivative glibenclamide has been shown to reduce hypoxia-induced vasodilation and adenosine, but not bradykinin, vasodilation as well. Nelson et al have suggested that the ATP-sensitive potassium channel may exert its effect on coronary vasomotor tone through its effect on the voltage-dependent calcium channel. The open-state probability of the voltage-dependent calcium channel depends on transmembrane voltage: at more negative hyperpolarized levels of transmembrane potential the channel is more likely to be closed, reducing the entry of calcium into the cell and resulting in vasodilation; at less negative, more depolarized levels of potential the channel is more likely to be open, increasing the entry of calcium into the cell and resulting in vasoconstriction.

Importantly, the ATP-sensitive potassium channel can influence transmembrane potential depending on whether its open-state probability is high or low. When ATP levels are high within the cell, the channel is usually closed and the cell is relatively depolarized; when ATP levels fall (e.g., during ischemia), the channel opens and the cell is relatively hyperpolarized.

We hypothesized that coronary occlusion results in opening of the ATP-sensitive potassium channel, pos-
sibly because of a decline in smooth muscle cell ATP levels. This, in turn, results in hyperpolarization of the smooth muscle cell and a reduction in the entry of calcium into the cell through voltage-dependent channels and, therefore, to a reduction of vasomotor tone. Blockade of the ATP-sensitive potassium channel may minimize the reactive hyperemia of myocardium because hyperpolarization will be prevented and, therefore, the open-state probability of the voltage-dependent calcium channel will be greater, leading to relative vasoconstriction or, in the case of reactive hyperemia, minimal reduction in vasomotor tone.

Materials and Methods

Eight 25-30-kg mongrel dogs were anesthetized with intravenous sodium thiopental (12.5 mg/kg body wt) and intramuscular chloralose (100 mg/kg body wt) in urethane, intubated, and ventilated with a piston respirator on supplemental oxygen. A thoracotomy was performed in the left fifth intercostal space, the pericardium was incised, and the heart was suspended in a pericardial cradle. A miniaturized, solid-state pressure transducer (MikroTip model PC-350, Millar Instruments, Inc., Houston, Tex.) was advanced into the left ventricle through the left atrium for measurement of left ventricular pressure. Fluids and drugs were administered as required via a catheter placed in a femoral vein. Pacemaker leads were attached to the left atrial appendage, and the heart was paced when required (see below). Heparin (10,000 IU) was administered intravenously.

The circumflex coronary artery was dissected, cannulated with a 3-mm steel cannula, and perfused via an extracorporeal circuit. For this circuit, the left subclavian artery was cannulated with a large-bore plastic tube and blood was pumped from this artery into a heated reservoir. Pressure in the circumflex artery was determined by controlling the pressure of blood in the reservoir by using compressed air. The circumflex cannula was fitted with a side arm that allowed measurement of circumflex pressure by a Statham P23-ID pressure transducer (Gould Statham); an in-line electromagnetic flow probe (BLC-2000, Biotronix Laboratory) allowed for measurement of coronary blood flow with a gated sinewave flowmeter (BL-613, Biotronix Laboratory).

Measurements

The duration of reactive hyperemia was defined as the time between the release of the coronary occlusion and the return of coronary flow to preocclusion flow. Flow debt was defined as preocclusion baseline flow rate times the duration of occlusion, debt repayment was defined as the area under the flow versus time curve during reactive hyperemia minus preocclusion baseline flow times the duration of reactive hyperemia, and percent debt repayment was defined as debt repayment divided by flow debt multiplied by 100. Coronary conductance was defined as mean coronary flow divided by mean coronary pressure. This definition is used with full recognition that it serves only as a relatively crude index of coronary conductance because “mean” outflow pressure is unmeasurable and the relation between coronary flow and pressure is curvilinear.

Materials

One millimole of glibenclamide (Sigma Chemical Co., St. Louis, Mo.) was dissolved in 1.5 ml dimethyl sulfoxide and then in 1,000 ml normal saline to which 30 meq sodium bicarbonate was added. A vehicle was made in identical fashion except for the elimination of glibenclamide.

Protocol

The study began at least 15 minutes after cannulation of the coronary artery.

Intracoronary infusion of vehicle was begun at 1.91 ml/min (n=5) or 3.68 ml/min (n=3). A 30-second reactive hyperemia was then recorded no sooner than 10 minutes after the start of the infusion. In three animals, a 60-second reactive hyperemia was then measured at least 5 minutes later. Peak coronary conductance was measured after graded intracoronary bolus doses of adenosine and acetylcholine, and dose-response curves were constructed.

The same measurements were obtained after a minimum of 10 minutes of administration of 0.8 and 3.7 μmol/min of intracoronary glibenclamide. If heart rate fell during glibenclamide administration, the heart was paced via the left atrium to maintain the heart rate near the preglibenclamide rate.

In three animals the ATP-sensitive potassium channel opener BRL34915 (cromakalim, a gift from Dr. T.C. Hamilton, Beecham Pharmaceutical Research Division, Harlow, Essex, England) was given after glibenclamide administration as a specific antagonist, and coronary flow and conductance were recorded.

Statistics

Data are expressed as mean ± SEM. Significant differences between two means were decided by paired t tests corrected for multiple comparisons by the Bonferroni method; differences among three means were decided by repeated measures analysis of variance. A level of p<0.05 was accepted as significant.

Results

Hemodynamics

Baseline heart rate was 124±8 beats/min, systolic left ventricular pressure was 96±6 mm Hg, mean left circumflex coronary artery pressure was 97±3 mm Hg, and the heart rate/systolic blood pressure product was 11,925±1,235 mm Hg/min. These parameters did not change significantly throughout the experiment.

Coronary Flow

Control resting coronary blood flow and coronary conductance were not changed significantly by glibenclamide infusion. As a result, flow debt was also unchanged.
Maximum coronary conductance and the duration of reactive hyperemia after 30-second inflow occlusions were reduced in a dose-dependent fashion by glibenclamide (Figure 1). As a result, reactive hyperemic flow and percent flow debt repayment were markedly reduced as illustrated in Figure 2 for one animal and summarized in Figure 3 for all animals. Similar results were noted in three animals after 60-second periods of occlusion.

Response to Vasodilators

The effect of intracoronary boluses of adenosine and acetylcholine on coronary conductance before and after glibenclamide administration is shown in Figure 4. While glibenclamide reduced the increase in coronary conductance after administration of intracoronary adenosine, there was no shift of the dose–response curve to intracoronary acetylcholine.

In three animals, the specific ATP-sensitive potassium channel opener, BRL34915, was administered after the high-dose glibenclamide infusion. As shown for one such animal in Figure 3, BRL34915 vasodilated the coronary circulation exposed to glibenclamide. This vasodilation could be reversed by additional bolus doses of glibenclamide.

Discussion

This study shows that in the coronary circulation of open-chest dogs with acute coronary occlusion, blockade of the ATP-sensitive potassium channel 1) reduces the duration of reactive hyperemia, 2) reduces maximum conductance during reactive hyperemia, 3) reduces the percent flow debt repayment after 30- and 60-second occlusions, and 4) reduces the sensitivity of the coronary vasculature to the vasodilator action of adenosine, but 5) does not change the overall vascular response to acetylcholine.

Experimental Limitations

Reactive hyperemia has been shown to depend on a number of factors including metabolic rate\(^1\) and perfusion pressure.\(^10\) Because heart rate and systolic blood pressure did not change and because coronary perfusion pressure was held nearly constant, it is not likely that either of these factors affected the results of this study. The time between occlusions can also affect flow debt repayment\(^11\) but was sufficiently long (at least 5 minutes) to obviate this potential problem.

In vitro studies of the ATP-sensitive potassium channel use a dose of glibenclamide in the 1–10 \(\mu\)M range. In our case, giving 0.8–3.7 \(\mu\)mol/min i.c. could result in a concentration as high as 80 \(\mu\)M. However, glibenclamide is highly protein bound\(^12\) (90% or more) so that its
actual concentration is probably within the range typically studied in in vitro experiments.

Other Studies

The existence of the ATP-sensitive potassium channel in the cardiac myocyte was first reported by Noma and coworkers. More recently, this channel has been reported to exist in vascular smooth muscle cells. Under normal conditions, the open-state probability of this channel is thought to be very low, at least in the cardiac myocyte, since ATP levels are quite high. Ischemia opens these channels as ATP levels fall. Channel opening hyperpolarizes the sarcolemmal membrane and leads to a reduction in calcium influx through voltage-dependent calcium channels. Thus, the ATP-sensitive potassium channel has been thought to serve a "protective role" in myocardial ischemia, perhaps minimizing calcium influx and resulting myocyte damage and arrhythmias after ischemia. Recent reports suggest that this, indeed, appears to be the case.

In the isolated guinea pig heart, blockade of the ATP-sensitive potassium channel has been shown to inhibit hypoxia-induced vasodilation and to reduce adenosine but not bradykinin vasodilation. Our study is the first to show marked reduction in the reactive hyperemic response in the intact, blood-perfused coronary circulation.

Remaining Questions

Because whole myocyte ATP levels probably do not fall significantly during a 30-second occlusion, it is unclear why blocking the ATP-sensitive potassium channel could reduce the reactive hyperemia after such a brief coronary occlusion. In cardiac myocytes, recent evidence suggests that during hypoxia the rapid fall in creatine phosphate and rise in ADP could activate the ATP-sensitive potassium channel before a significant decline in ATP is detectable; perhaps the same is true of the vascular smooth muscle cell. The relation between ADP and opening of the ATP-sensitive potassium channel is complex and dependent on intracellular magnesium levels: at low levels of magnesium, ADP actually inhibits the channel, whereas in the presence of physiological intracellular magnesium levels, a rise in ADP level increases channel opening at any level of ATP. In addition, it is possible that although ATP levels do not fall in the cardiac myocyte during a 30-second occlusion, they do fall in the vascular smooth muscle cell, perhaps because ATP and phosphocreatinine

FIGURE 3. Effect of glibenclamide (glyburide) on percent flow debt repayment. *p<0.05 compared with control. Difference between flow debt repayment at glibenclamide doses of 0.8 and 3.7 μmol/min did not reach statistical significance.

FIGURE 4. Dose-response curves to adenosine (upper panel) and acetylcholine (lower panel) before (■—■) and after (○—○) high-dose glibenclamide. Note that the adenosine dose–response curve was shifted downward and to the right. With acetylcholine no overall change in response was noted. In the adenosine graph, the number of dogs represented in each data point from the lowest to the highest adenosine dose was three, four, seven, eight, and six before glibenclamide; and two, three, four, eight, six, and five after glibenclamide. In the acetylcholine graph, the number of dogs represented in each data point from the lowest to the highest acetylcholine dose was three, six, four, four, and three before glibenclamide; and three, six, five, four, and three after glibenclamide. *p<0.05 compared with after glibenclamide.
levels are lower in this tissue than in the cardiac myocyte. Furthermore, ATP may be compartmentalized so that only the ATP level existing just within the plasma membrane and determined mainly by glycolysis is important in modulating the function of the plasma membrane-associated ATP-sensitive potassium channel. In addition, there are a large number of other factors that either modulate the effect of ATP on this channel or affect the channel directly.

Conclusions

Blockade of the ATP-sensitive potassium channel, probably through its effect on transmembrane potential and the voltage-sensitive calcium channel, appears to be an important determinant of reactive hyperemia. That reactive hyperemia was not completely eliminated suggests that factors other than the ATP-sensitive potassium channel may affect the voltage-dependent calcium channel during coronary occlusion. For example, stretch of the smooth muscle cell in the vasculature may change resting membrane potential and affect ion flux through voltage-dependent calcium channels.

The results of this and other studies focus the attention of the coronary physiologist on membrane ion channels as potentially important in the regulation of coronary blood flow. Which factors modulate these channels and, therefore, determine vasomotor tone is an important area for future study.

Acknowledgment

The expert technical assistance of Mr. Alexander Wright is gratefully acknowledged.

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Key Words • reactive hyperemia • ATP-sensitive potassium channels • voltage-dependent calcium channels • coronary blood flow
Blockade of the ATP-sensitive potassium channel modulates reactive hyperemia in the canine coronary circulation.

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doi: 10.1161/01.RES.69.3.618

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/69/3/618

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