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Activation of ATP-sensitive potassium channels contributes to reactive hyperemia in humans

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Banitt, Peter F., Paul Smits, Stephen B. Williams, Peter Ganz, and Mark A. Creager. Activation of ATP-sensitive potassium channels contributes to reactive hyperemia in humans. Am. J. Physiol. 271 (Heart Circ. Physiol. 40): H1594–H1598, 1996.—Activation of ATP-sensitive potassium (K_{ATP}) channels present on vascular smooth muscle cells causes membrane hyperpolarization and vasodilation. The purpose of this study was to determine whether K_{ATP} channels contribute to reactive hyperemia in humans. Accordingly, we studied the effect of tolbutamide, a K_{ATP} channel inhibitor, on reactive hyperemic forearm blood flow. Forearm blood flow was measured by venous occlusion plethysmography. Forearm ischemia was produced by inflating a sphygmomanometric cuff on the arm to suprasystolic pressures for 5 min. After cuff release, forearm blood flow was measured during the reactive hyperemic phase for 5 min. Tolbutamide (1 mM blood concentration, n = 6) did not affect basal (2.4 ± 0.2 to 2.2 ± 0.1 ml·100 ml·min^{-1}) or peak reactive hyperemic forearm blood flow (21.9 ± 3.8 to 22.6 ± 2.9 ml·100 ml·min^{-1}, each P = NS), but it significantly attenuated total hyperemic volume (12.6 ± 1.7 vs. 9.2 ± 1.8 ml/100 ml, P < 0.02). Vehicle (n = 6) did not affect basal flow, peak reactive hyperemic flow, or total hyperemia. To determine whether adenosine or endothelium-derived nitric oxide contribute to reactive hyperemia via K_{ATP} channels, adenosine (1.5–500 μg/min, n = 6) and acetylcholine (30 μg/min, n = 6) were infused before and during tolbutamide coinfusion. Tolbutamide did not significantly alter the forearm blood flow response to either adenosine or acetylcholine. In conclusion, K_{ATP} channels contribute to vasodilation during reactive hyperemia in humans.

Methods

Subjects. Eighteen healthy volunteers were recruited to participate in this study. The study subjects included nine males and nine females, 22–45 years of age (averaging 32.6 ± 1.5 yr). Each volunteer's health status was assessed by history, physical examination, and laboratory analysis of serum lipid and glucose levels. None of the subjects had clinical evidence of systemic disease, and none used tobacco products. This study was approved by the Human Research Committee of Brigham and Women's Hospital, and each subject gave written informed consent.

General procedures. Each subject was studied in the supine position in a 23°C temperature-controlled room in the postabsorptive state. Alcohol and caffeine were not permitted within the preceding 12 h. Using sterile technique and local anesthesia, we inserted a 20-gauge polyethylene catheter into the brachial artery for blood pressure monitoring and drug infusion. The vascular research laboratory was quiet, and lights were dimmed. All subjects rested at least 30 min after catheter placement to establish a stable baseline before data acquisition.

Hemodynamic measurements. Bilateral forearm blood flow was determined by venous occlusion strain-gauge plethysmography using calibrated mercury-in-Silastic strain gauges (D. E. Hokanson, Issaquah, WA) and is expressed as milliliter per 100 milliliters tissue per minute. Each arm was supported above heart level. The venous occlusion pressure was 40 mmHg. Hand blood flow was prevented during measurements by inflating a cuff at the wrist to suprasystolic levels. Each forearm blood flow determination consisted of at least five separate measurements performed at 10- to 15-s inter-
Forearm vascular resistance was calculated as the ratio of mean blood pressure to forearm blood flow and expressed as units reflecting millimeter Hg per milliliter per 100 ml tissue per minute. Blood pressure was recorded via the arterial cannula attached to a pressure transducer and was recorded on a physiological recorder (Gould, Cleveland, OH). Heart rate was determined from a simultaneously obtained electrocardiographic signal and calculated from the R-R interval.

**Experimental protocols.** Reactive hyperemia was produced by inflating a cuff on the upper arm to suprasystolic pressures for 5 min and then releasing it. Blood flow measurements were recorded as quickly as possible during the first minute following cuff deflation (approximately every 3–5 s) to accurately assess the peak reactive hyperemic response. Peak reactive hyperemic blood flow was chosen as the highest flow recorded during this time. During the second and third minutes following cuff release, blood flow measurements were obtained every 15 s, and during the fourth and fifth minutes after cuff deflation, measurements were taken every 30 s. Total hyperemia was determined by calculating the area under the flow versus time curve and subtracting the baseline flow and expressed as milliliter per 100 ml tissue.

To assess whether the increase in flow during reactive hyperemia was due to KATP channel activation, measurements were obtained in six subjects at baseline and during reactive hyperemia, before and during intra-arterial administration of the KATP channel inhibitor tolbutamide. The tolbutamide infusion was adjusted for each individual after baseline forearm blood flow and forearm volume were measured, to achieve a final arterial concentration of tolbutamide of 1 mM. Tolbutamide was infused into the brachial artery at a rate of 0.4 ml/min for 10 min via a syringe infusion pump (Harvard Apparatus, South Natick, MA). Blood for serum glucose and insulin levels was drawn immediately before each reactive hyperemia, i.e., before and during the tolbutamide infusion.

To determine whether nitric oxide could have been responsible for activation of KATP channels during reactive hyperemia, we measured the forearm blood flow response to acetylcholine (an endothelium-dependent vasodilator that causes nitric oxide release) at a dose of 30 μg/min administered via the brachial artery before and during tolbutamide infusion in the same six subjects.

To assure ourselves that the inhibitory effects of tolbutamide on reactive hyperemic blood flow were only related to KATP channel inhibition and not a consequence of time and repeated measuring, reproducibility experiments were performed in a separate group of six subjects. The reactive hyperemia protocol was performed, except that vehicle was administered instead of tolbutamide.

To determine whether adenosine could have been responsible for activation of KATP channels observed during reactive hyperemia, the effect of tolbutamide on adenosine-induced vasodilation was studied in six additional normal subjects. Increasing doses of adenosine (1.5, 5, 15, 50, 150, and 500 μg/min) were infused via the brachial artery every 4 min. After the adenosine infusion was stopped, a 30-min drug washout period followed. Tolbutamide (1 mM) was then administered at 0.4 ml/min for 10 min. Adenosine (1.5–500 μg/min) was then infused with tolbutamide. Forearm blood flow and blood pressure were recorded during each stage of the protocol.

**Drugs.** Tolbutamide sodium (Orinase Diagnostic) was obtained from Upjohn (Kalamazoo, MI) and was freshly prepared for each experiment by diluting in 0.9% NaCl. Acetylcholine chloride (Miochol) was obtained from Iolab (Claremont, CA) and was freshly prepared by diluting in 0.9% NaCl. Adenosine was also prepared for each experiment by diluting Adenocard vials (6 mg/2 ml, Fujisawa Pharmaceutical, Deerfield, IL) into saline.

**Statistical analysis.** Results are presented as means ± SE. All paired data sets were compared using the two-tailed Student’s t-test. Comparisons between adenosine dose-response curves were performed using two-way analysis of variance with repeated measures. A P value ≤0.05 was considered to be statistically significant.

**RESULTS**

**Effect of tolbutamide on reactive hyperemic blood flow.** Under control conditions, basal forearm blood flow was 2.3 ± 0.2 ml·100 ml−1·min−1, mean blood pressure was 76 ± 2 mmHg, and forearm vascular resistance was 34 ± 3 units. After release of a 5-min arterial occlusion, there was an immediate increase in forearm blood flow and decrease in forearm vascular resistance, which gradually returned to baseline over 2–4 min. Peak reactive hyperemic blood flow was 21.9 ± 3.8 ml·100 ml−1·min−1, minimal forearm vascular resistance was 4.0 ± 0.8 units, and total hyperemic volume was 12.6 ± 1.7 ml/100 ml. Intra-arterial infusion of tolbutamide did not affect mean blood pressure (to 77 ± 3 mmHg, P = NS), basal forearm blood flow (to 2.2 ± 0.4 ml·100 ml−1·min−1, P = NS), or peak reactive hyperemic flow (to 22.6 ± 2.9 ml·100 ml−1·min−1, P = NS) (Fig. 1). However, tolbutamide significantly reduced the total hyperemic volume by 27% to 9.2 ± 1.8 ml/100 ml (P < 0.02) (Fig. 2). The time until the decrease in flow reached 50% of the peak flow value for hyperemia (t1/2) was shortened by 36% from 35.2 ± 6.4 to 22.4 ± 3.5 s (P = 0.05).

Infusion of vehicle did not affect basal forearm blood flow (2.5 ± 0.2 vs. 2.6 ± 0.3 ml·100 ml−1·min−1, P = NS), peak reactive hyperemic blood flow (22.6 ± 1.2 vs. 26.0 ± 2.2 ml·100 ml−1·min−1, P = NS), or total hyperemic volume (11.4 ± 1.1 vs. 13.9 ± 1.1 ml/100 ml, P = NS) (Figs. 1 and 2).

Serum glucose levels were monitored during the tolbutamide infusion and did not change compared with baseline (77 ± 3 to 78 ± 4 mg/dl, P = NS). Similarly, serum insulin levels were not affected by the

**Fig. 1.** Effect of tolbutamide and vehicle on peak reactive hyperemic blood flow (RHBF). No significant changes were observed with either intervention.
intra-arterial tolbutamide infusion (4.2 ± 1.2 vs. 5.9 ± 0.7 μU/ml, P = NS).

**Effects of tolbutamide on adenosine and acetylcholine-induced vasodilation.** Adenosine (1.5–500 μg/min) increased forearm blood flow incrementally from a basal value of 2.0 ± 0.3 to 15.2 ± 2.7 ml·100 ml⁻¹·min⁻¹. Tolbutamide did not affect the forearm blood flow response to adenosine (Fig. 3). Acetylcholine (30 μg/min) increased forearm blood flow from 2.5 ± 0.2 to 14.0 ± 2.8 ml·100 ml⁻¹·min⁻¹ and decreased forearm vascular resistance from 34.3 ± 2.8 to 7.3 ± 2.2 units. The vasodilator effects of acetylcholine were not inhibited by tolbutamide (Fig. 4).

**DISCUSSION**

The important new finding in this study is that tolbutamide, a K<sub>ATP</sub>-channel inhibitor, attenuates reactive hyperemic blood flow in the human forearm. Tolbutamide did not affect peak reactive hyperemic blood flow, but it did significantly reduce total hyperemic volume. Additionally, we found that adenosine-induced vasodilation is not inhibited by tolbutamide infusion, indicating that adenosine does not activate K<sub>ATP</sub> channels during reactive hyperemia. Finally, tolbutamide did not affect vasodilation in response to the endothelium-dependent vasodilator acetylcholine, suggesting that endothelium-derived nitric oxide does not activate K<sub>ATP</sub> channels.

**Vascular smooth muscle membrane hyperpolarization.** Vascular smooth muscle tone is regulated by intracellular calcium concentrations (17). Important determinants of intracellular calcium regulation are voltage-dependent calcium channels that respond to changes in transmembrane potential of vascular smooth muscle cells. Three types of potassium channels may induce membrane hyperpolarization and thereby affect voltage-dependent calcium channels: calcium-activated potassium channels, delayed rectifier potassium channels, and K<sub>ATP</sub> channels.

In 1983, Noma (28) first reported the presence of potassium-selective ion channels that were inhibited by normal cytosolic concentrations of ATP. In ATP-depleted states, such as myocyte ischemia, these channels are activated, resulting in membrane hyperpolarization. Sulfonylurea compounds such as tolbutamide and glibenclamide (16, 29, 30) have been shown to specifically inhibit K<sub>ATP</sub> channels. In this study, tolbutamide was chosen to block the effects of K<sub>ATP</sub> channels because its parenteral form is available for use in humans. In vitro studies have demonstrated that tolbutamide in the range of 1 mM inhibits K<sub>ATP</sub> channels and blocks vasodilation (15, 30). Therefore, we adjusted our intra-arterial tolbutamide infusions such that the net concentration of tolbutamide in the brachial artery was 1 mM. Protein binding may have decreased the free tolbutamide concentration (2); however, pilot studies with higher tolbutamide infusion concentrations resulted in hypoglycemia.

**Contribution of K<sub>ATP</sub> channels to reactive hyperemia.** Evidence derived from experimental animal models supports the hypothesis that K<sub>ATP</sub> are involved in regulating vascular smooth muscle tone during reactive hyperemia. Aversano et al. (1) reported that glibenclamide administered into the circumflex coronary artery of open-chest anesthetized dogs decreased reactive
hyperemia by 50% after a 30-s coronary occlusion. Duncker et al. (14) made similar observations in conscious dogs. Kanatsuka et al. (20), using a floating objective microscope to observe canine coronary epicardial microvessels, reported that intracoronary glibenclamide (200 μg/kg) significantly blunted microvascular vasodilation after a 30-s coronary occlusion. This attenuation of vasodilation was most marked in vessels <100 μm in diameter but was present in all vessels studied. Similarly, canine diaphragmatic reactive hyperemic flow and duration is also significantly blunted by glibenclamide (8 × 10⁻⁵ M) (33). In addition to reactive hyperemia, K<sub>ATP</sub> channels have been shown to mediate the vasodilation seen in response to other stimuli such as hypoxia and decreased perfusion pressure. Using an isolated guinea pig heart model, Daut et al. (12) demonstrated that hypoxia-induced coronary artery vasodilation is prevented by glibenclamide. Similarly, using an isolated rabbit heart model, Nakhostine and Lamon-tagne (27) observed that glibenclamide significantly attenuated hypoxia-induced vasodilation. Komaru et al. (23) reported that topical glibenclamide abolished vasodilation of epicardial arteries during periods of reduced perfusion pressure.

In this study we report that K<sub>ATP</sub> channels also are operative in the human vasculature, because tolbuta-mide significantly blunted the total reactive hyperemic flow. The degree of total reactive hyperemia inhibition (27% compared with control) is somewhat less than that observed in many of the animal models cited above.

Adenosine-induced vasodilation. Many investigators have suggested that adenosine is a mediator of vasodila-tion during reactive hyperemia (5, 7, 13, 35). Evi-dence that adenosine can directly activate K<sub>ATP</sub> chan-nels has been found in some, but not all, studies (3, 11, 18, 24, 27). We sought to determine whether the decrease in total reactive hyperemic flow during tolbuta-mide infusion implicated a reduction in adenosine-induced K<sub>ATP</sub> channel activation. We found that tolbuta-mide did not inhibit forearm vasodilation to adenosine; this suggests that the effects of adenosine are not mediated via K<sub>ATP</sub> channels in human forearm resistance vessels. The lack of effect of tolbutamide on adenosine-induced vasodilation does not eliminate adenosine as a participant in reactive hyperemia because adenosine can cause vasodilation by other mechanisms, such as direct activation of adenosine receptors on vascular smooth muscle.

Endothelium-dependent vasodilation. The vascular endothelium can elaborate a number of potent vasoco-rticular substances, including nitric oxide and a yet uniden-tified hyperpolarizing factor (6, 9, 10, 26, 32). Previous studies in humans have demonstrated that nitric oxide inhibition with N<sup>ω</sup>-monomethyl-L-arginine (1-NMMA) reduces total reactive hyperemia (19, 25, 31). Most evidence suggests that the action of nitric oxide is via activation of guanylyl cyclase and increased production of guanosine 3′,5′-cyclic monophosphate; however, nitric oxide also may cause vasodilation through vascular smooth muscle hyperpolarization (32). Endothelium-dependent hyperpolarizing factor hyperpolarizes the vascular smooth muscle and results in vasodilation (6, 9, 10, 26, 32). Some, but not all, studies suggest that K<sub>ATP</sub> channels mediate this hyperpolarization (6, 9, 10, 29). This study does not directly assess whether the endothelium is involved in vascular smooth muscle hyperpolarization. However, the fact that acetylcholine-induced vasodilation is not affected by tolbutamide suggests that nitric oxide release does not activate K<sub>ATP</sub> channels in the human forearm.

Physiological implications. In this study, tolbuta-mide infusion did not affect peak reactive hyperemic flow, suggesting that in the human forearm the mecha-nisms causing maximal vasodilation following an ischemic stimulus are not dependent on K<sub>ATP</sub> channel activation. However, tolbutamide did attenuate total reactive hyperemia, indicating that K<sub>ATP</sub> channel activation contributes to vasodilation during the sustained phase of reactive hyperemia.

Other factors are involved in mediating reactive hyperemic blood flow in humans, including nitric oxide, prostaglandins, and adenosine; the relative contribu­tion of these factors may vary from the peak to the sustained phase of reactive hyperemia. We have found previously that nitric oxide contributes to peak and total reactive hyperemic blood flow, but others have reported that nitric oxide is involved only in the sus-tained response (19, 25, 31). Inhibition of prostaglandin production with cyclooxygenase inhibitors has been reported to blunt peak reactive hyperemic flow in humans in two studies (7, 21) but not in another (19). When these observations are taken together, it is apparent that a number of mediators contribute to reactive hyperemia in human limb resistance vessels. The relative contribution of each in human health and disease is not known and must await additional investiga­tion.

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