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Acute effects of glyburide on the regulation of peripheral blood flow in normal humans

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Abstract

Recent animal studies have demonstrated that selective blockade of ATP-sensitive K^+ (K_{ATP}) channels of vascular smooth muscle results in a significant increase in peripheral vascular tone. The main aim of this study was to assess whether glyburide, a selective blocker of K_{ATP} channels and commonly used antidiabetic agent, influences resting blood flow and reactive hyperemic response of peripheral tissues of normal subjects. Baseline calf blood flow was measured non-invasively in six normal subjects with femoral venous occlusive plethysmography. Calf blood flow was also serially measured every 30–60 s after the release of calf arterial occlusion (10 min duration). Reactive hyperemia was expressed in terms of peak post-occlusive flow, duration of hyperemia and reactive hyperemic volume. In each subject, baseline flow and reactive hyperemia were measured before (control) and every hour for 5 h after the oral ingestion of either 7.5 mg glyburide or a placebo on two separate days. Baseline calf flow declined by 30 and 42% of control values after 1 and 2 h of glyburide intake ($P < 0.05$) with a return to control values by hours 3, 4 and 5. Peak post-occlusive flow after 1, 2 and 3 h of glyburide ingestion was lower than control values by 22, 30 and 28%, respectively ($P < 0.05$). The duration of reactive hyperemia after 2 and 3 h of glyburide ingestion was significantly longer than control values ($P < 0.05$), whereas reactive hyperemic volume remained unaffected by glyburide intake. Placebo elicited no significant changes in baseline flow or reactive hyperemia throughout the 5-h experimental period. These results indicate that a single dose of glyburide in the therapeutic range elicits significant alterations in the regulation of peripheral blood flow in normal subjects. We propose that these alterations are mediated through the blockade of vascular smooth muscle K_{ATP} channels.

Keywords: Blood vessel; Hyperemia; Vascular control; K^+ channel

1. Introduction

Peripheral blood flow is determined by changes in perfusion pressure and local vascular resistance. The latter is influenced by locally released and circulating vasoactive substances and autonomic neural drive (Olsson, 1989). In addition, several locally released endothelium-derived vasorelaxants, particularly prostaglandins and nitric oxide, have been identified and their role in the regulation of blood flow in several vascular beds has been described (Carlsson et al., 1987; Förstermann et al., 1987). The contribution of these locally released regulators of blood flow is substantial compared with systemic factors.

Recently, transmembrane potential of vascular smooth muscle cells has been proposed to be an important additional element in the local regulation of vascular tone. The effect of membrane potential is theorized to be mediated by the modulation of Ca^{2+} influx through voltage-dependent vascular smooth muscle Ca^{2+} channels (Lombard et al., 1984). Among various ionic channels that are involved in the regulation of vascular smooth muscle membrane potentials are ATP-sensitive potassium channels (K_{ATP}) which have been identified in vascular smooth muscle cells (Cavero et al., 1989; Standen et al., 1989; Nichols and Lederer, 1991). When these channels are open, K^+ efflux increases leading to hyperpolarization of membrane potential and consequently to a reduction in Ca^{2+} influx

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and to smooth muscle relaxation. Activation of these channels has recently been shown to contribute to basal coronary vascular tone and to ischemic and hypoxic coronary vasodilation (Daut et al., 1990; Imamura et al., 1992; Samaha et al., 1992).

Glyburide, a second-generation sulfonylurea, is commonly used in the treatment of non-insulin dependent diabetes mellitus (Feldman, 1985). Its mechanism of action is believed to be mediated through binding of sulfonylurea receptors of pancreatic β-cells (Boyd, 1988). Activation of these receptors is associated with the closure of K_{ATP} channels and reduction in the efflux of potassium ions. As a result, membrane depolarization develops which leads to increased Ca^{2+} influx and increased insulin release by pancreatic β-cells (Boyd et al., 1990). Sulfonylureas are generally well tolerated drugs; however, a serious consideration in treating patients with a sulfonylurea agent is the reported high incidence of cardiovascular mortality as compared with treatment with diet alone (Jackson and Bressler, 1981; University Group Diabetes Program, 1970). The observations that K_{ATP} channels exist in the vascular smooth muscles and that selective blockade of these channels with glyburide is associated with a significant increase in resting vascular tone and reduction in hypoxic vasodilation raises the possibility that glyburide therapy may be associated with impairment of peripheral and cardiac blood flow and, thereby, contribute to this increase in mortality. In spite of this possibility, no study has yet evaluated the effects of glyburide therapy on the regulation of peripheral blood flow in normal humans.

The main aim of this study was, therefore, to assess whether the acute administration of glyburide at a therapeutic dosage influences the control of peripheral blood flow in normal subjects. We measured the changes in resting calf blood flow and calf reactive hyperemia throughout a 5-h experimental period after the ingestion of 7.5 mg of glyburide. A similar protocol was repeated after the ingestion of a placebo.

2. Materials and methods

2.1. Subjects

Six healthy male subjects aged 27–40 years volunteered for the study. All subjects were sedentary, in good health and gave no prior medical history. The study protocol was approved by the Institutional Ethics Committee.

2.2. Baseline flow

Calf blood flow was measured non-invasively after rapid inflation of a thigh tourniquet cuff to subdiastolic pressure (50 mm Hg) with the technique of femoral venous occlusive plethysmography (Holling et al., 1961; Hiatt et al., 1989). The accuracy and reproducibility of this technique have been discussed elsewhere (Holling et al., 1961; Hiatt et al., 1989). Serial measurements of calf arterial inflow were determined using a self-balancing and electrically calibrated mercury strain gauge plethysmograph (EC-4 Plethysmograph, D.E. Hokanson, Bellevue, WA, USA). Calf volume changes produced proportional elongations of a mercury-in-silastic transducer, which was positioned around the widest part of the calf. The transducer was connected to a strip-chart recorder (Hewlett-Packard 7758B System, Boston, MA, USA) and its length alterations produced a signal from which calf blood flow was calculated by drawing a tangent line to the initial part of the slope of the volume vs. time plot displayed on the recording paper. The foot was isolated hemodynamically from the calf circulation by the inflation of an ankle tourniquet cuff to 250 mm Hg. Blood flow measurements were made in the supine position with the leg raised slightly to facilitate venous drainage between flow measurements. Baseline calf flow was estimated as the mean of three measurements. Blood pressure measurements were obtained every minute from a brachial artery using a sphygmomanometer. Baseline calf vascu-

Table 1
Changes in baseline calf blood flow, peak post-occlusive calf flow, duration of reactive hyperemia and total reactive hyperemic volume in healthy subjects before (control) and within 5 h of the adminstration of 7.5 mg glyburide

Time (h)	Baseline flow (ml/min/100 ml)	Peak post-occlusive flow (ml/min/100 ml)	Duration of reactive hyperemia (min)	Reactive hyperemic volume (ml/100 ml)
Control	4.23 ± 0.58	36.45 ± 3.44	3.17 ± 0.37	24.60 ± 3.69
1	2.69 ± 0.25 *	27.98 ± 1.56 *	3.83 ± 0.44	24.02 ± 3.51
2	2.20 ± 0.17 *	25.33 ± 2.08 *	4.67 ± 0.19 *	26.18 ± 2.63
3	3.02 ± 0.34	24.99 ± 2.38 *	5.00 ± 0.24 *	27.25 ± 2.25
4	3.66 ± 0.50	28.82 ± 4.36	3.83 ± 0.15	22.83 ± 3.99
5	4.05 ± 0.50	34.12 ± 4.55	3.51 ± 0.20	22.00 ± 4.45

Values are means ± S.E. * $P < 0.05$ compared with control values.

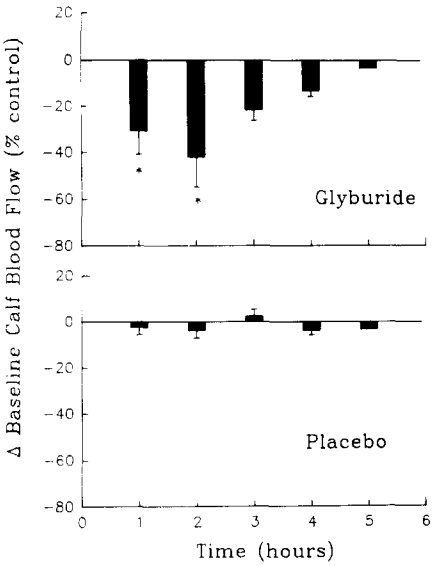

Fig. 1. The changes in baseline calf blood flow (percentage of control) over a 5-h period after the ingestion of a single oral dose of glyburide (top) or placebo (bottom). $^{*}P < 0.05$ compared with control values. Note the significant decline in calf flow 1 and 2 h after glyburide intake.

lar resistance was calculated as the ratio of mean arterial blood pressure and baseline calf blood flow.

2.3. Reactive hyperemia

To study the effect of glyburide on the reactive hyperemic response, we measured calf blood flow in response to 10 min of femoral arterial occlusion achieved by inflating a thigh cuff to 250 mm Hg. Post-occlusive calf blood flow was measured after 30 s, 60 s and every minute after the release of femoral occlusion until the calf flow recovered to within 10% of baseline values. At each individual investigation, a blood flow-time curve was constructed. Reactive hyperemia duration was calculated as the time from the release of femoral arterial occlusion to the return of baseline calf flow. Reactive hyperemic volume for each occlusion period was calculated as the time integral of blood flow above baseline values.

2.4. Experimental protocol

Subjects underwent vascular studies on two separate days (2–7 days apart). On both days, the subjects had the same breakfast at around 9 a.m., then control measurements of baseline calf blood flow and reactive hyperemic response to 10 min arterial occlusion were performed. Subjects then ingested either glyburide (7.5 mg) or a placebo in a randomized order. Measurements of baseline calf flow and reactive hyperemia on both days were repeated every hour for the next 5 h. Venous blood was sampled every hour for glucose estimation. On the day of the experiment, the subjects were allowed normal daily activity and were encouraged to take regular meals.

2.5. Data analysis

All data are reported as means ± standard errors of the means. Data for baseline calf flow and reactive hyperemic parameters obtained after the intake of glyburide or placebo were compared to their respective control values using the two-way analysis of variance for repeated measures. P values of less than 0.05 were considered significant.

3. Results

3.1. Glyburide experiments

Control values of mean arterial pressure, baseline calf flow and baseline calf vascular resistance averaged 93.8 ± 2.3 mm Hg, 4.23 ± 0.58 ml/min/100 ml tissue and 24.6 ± 2.9 mm Hg/ml/min/100 ml tissue, respectively. Mean arterial pressure measured at 1, 2, 3, 4 and 5 h after glyburide intake was not different from control values (mean values of 92.4, 94.7, 93.8, 95.3 and 93.0 mm Hg, respectively). Baseline calf blood flow at 1 and 2 h after glyburide intake declined significantly lower than control values with a return to control values within 3 h (Table 1). The decline in baseline calf flow at 1 and 2 h after glyburide intake averaged 30

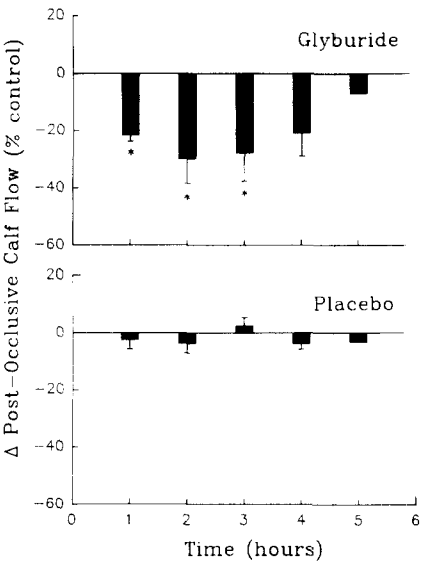

Fig. 2. Effects of glyburide (top) and placebo (bottom) intake on peak post-occlusive calf flow measured after a 10-min period of complete arterial occlusion. Values are expressed as percentage of peak post-occlusive calf flow measured during the control period. $^{*}P < 0.05$ compared with control values. Note the significant reduction in peak post-occlusive flow 1–3 h after glyburide intake.

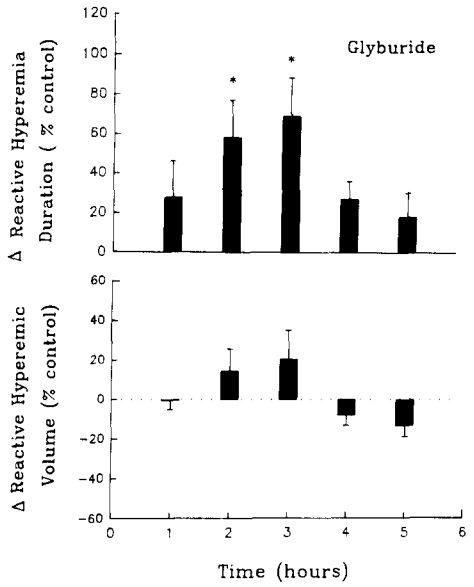

Fig. 3. The effects of glyburide on reactive hyperemia duration (top) and reactive hyperemic volume (bottom) measured after a 10-min period of complete calf arterial occlusion. $^{*}P < 0.05$ compared with control.

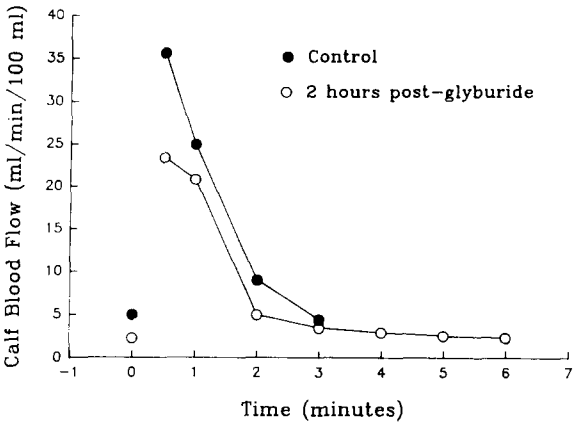

Fig. 4. The influence of glyburide on baseline calf flow and calf reactive hyperemia in a representative subject.

and 42% of control values, respectively (Fig. 1, top). Control values of blood glucose averaged 5.6 ± 0.3 mM/l. At 1, 2 and 3 h after glyburide intake, blood glucose declined slightly to 4.2 ± 0.5, 3.7 ± 0.1 and 3.8 ± 0.8 mM/l, respectively, with complete recovery to control values after 5 h (5.0 ± 0.05 mM/l)

During the control period, significant calf vascular dilation was evident after 10 min of complete ischemia. Peak post-occlusive calf flow, duration of reactive hyperemia and reactive hyperemic volume during this period averaged 36.45 ± 3.44 ml/min/100 ml of tissue, 3.17 ± 0.37 min and 24.60 ± 3.69 ml/100 ml of tissue, respectively. Peak post-occlusive calf flow at 1, 2 and 3 after glyburide intake declined significantly compared with control values (Table 1 and Fig. 2, top). By comparison, peak post-occlusive flow values measured at 4 and 5 h after glyburide intake were not different from control (Table 1 and Fig. 2). The duration of reactive hyperemia at 2 and 3 h after glyburide ingestion was significantly longer than control values (Table 1 and Fig. 3, top). Glyburide intake had no significant effect on reactive hyperemic volume measured throughout the experiment (Table 1 and Fig. 3, bottom).

Fig. 4 illustrates the changes in baseline calf flow, peak post-occlusive calf flow and duration of reactive hyperemia during the control period and at 2 h after glyburide intake in a representative subject.

3.2. Placebo experiments

Control values of mean arterial pressure, baseline calf flow and baseline calf flow and baseline calf vascular resistance averaged 96.0 ± 2.0 mm Hg, 4.12 ± 0.57 ml/min/100 ml of tissue and 25.8 ± 3.0 mm Hg/ml/min/100 ml of tissue, respectively. Mean arterial pressure measured at 1, 2, 3, 4 and 5 h after placebo intake was not different from control values (mean values of 95.0, 94.2, 93.2, 93.9 and 95.1 mm Hg, respectively). Placebo intake had no significant effect on baseline calf flow (Table 1 and Fig. 1, bottom). Significant calf vascular dilation was evident after 10 min of complete ischemia during the control period. Peak post-occlusive calf flow, duration of reactive hyperemia and reactive hyperemic volume during this period averaged 33.20 ± 3.50 ml/min/100 ml of tissue,

Table 2
Changes in baseline calf blood flow, peak post-occlusive calf flow, duration of reactive hyperemia and total reactive hyperemic volume in healthy subjects before (control) and within 5 h of the adminstration of placebo

Time hours	Baseline flow (ml/min/100 ml)	Peak post-occlusive flow (ml/min/100 ml)	Duration of reactive hyperemia (min)	Reactive hyperemic volume (ml/100 ml)
Control	4.12 ± 0.57	33.20 ± 3.50	3.33 ± 0.38	21.17 ± 2.43
1	3.92 ± 0.49	32.72 ± 2.37	3.50 ± 0.39	21.74 ± 2.81
2	3.85 ± 0.49	31.22 ± 3.01	3.83 ± 0.55	22.34 ± 2.58
3	3.96 ± 0.39	31.55 ± 2.40	3.50 ± 0.39	22.15 ± 2.83
4	3.81 ± 0.49	31.18 ± 3.00	3.65 ± 0.47	22.60 ± 2.69
5	3.92 ± 0.53	32.52 ± 3.17	3.33 ± 0.39	21.24 ± 2.63

Values are means ± S.E.

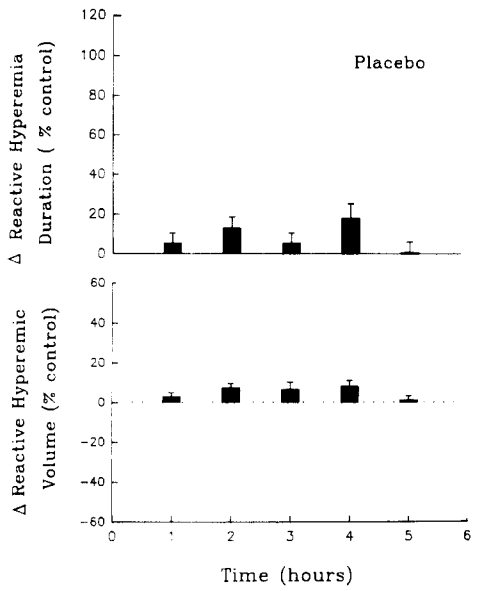

Fig. 5. The effects of placebo on reactive hyperemia duration and reactive hyperemic volume measured after 10-min periods of complete calf arterial occlusion. Placebo had no influence on reactive hyperemic duration and reactive hyperemic volume.

3.33 ± 0.38 min and 21.17 ± 2.43 ml/100 ml of tissue, respectively. Placebo intake resulted in no significant changes in peak post-occlusive flow, duration of reactive hyperemia and reactive volume (Table 2, and Figs. 2 and 5).

4. Discussion

The main finding of this study is that both baseline and peak post-occlusive calf flow declined significantly 1–3 h after the intake of a single dose of glyburide in normal subjects. Placebo had no effect on baseline or post-occlusive calf flow.

4.1. Mechanisms of action of glyburide

Glyburide is an effective second generation sulfonylurea used primarily in the treatment of non-insulin dependent diabetes mellitus. When taken as a single oral daily dose (1.25–20 mg), glyburide has been reported to be more potent in controlling blood glucose than first-generation sulfonylureas such as tolbutamide and acetohexamide (Gerich, 1989). On the other hand, the duration of glyburide action is relatively long, ranging between 18 and 24 h after a single oral dose (Gerich, 1989). Stimulation of insulin production by glyburide has been attributed mainly to its binding to sulfonylurea receptors of pancreatic β-cells which, in turn, leads to closure of K_{ATP} channels (Boyd et al., 1990). These channels have been identified in pancreatic β-cells and are known to set the resting membrane potential of these cells (Ashcroft and Kakei, 1989). Closure of K_{ATP} channels by glyburide causes membrane depolarization and augmentation of Ca^{2+} influx through voltage-dependent Ca^{2+} channels. K_{ATP} channels under normal conditions are closed through the effect of intracellular ATP; however, in the absence of glucose, opening of a small number of K_{ATP} channels leads to K^+ efflux and stabilization of membrane potential at a hyperpolarized state. Increased intracellular ATP (caused by augmentation of intracellular glucose concentration) leads to the closure of K_{ATP} channels and consequently results in depolarization of membrane potential and increased Ca^{2+} influx which, in turn, elicits insulin secretion. The exact mechanism through which glyburide inhibits K_{ATP} channels remains under investigation. Boyd et al. (1990) proposed that glyburide may either bind sulfonylurea receptors and K_{ATP} channels directly or alternatively may enter the membrane and move through the bilayer to a binding site near the membrane channel or on the inner surface near the ATP binding site.

4.2. Effect of glyburide on resting flow

Recently, substances released by the endothelium have been shown to play a major role in the regulation of peripheral blood flow. These include prostaglandins and endothelium-derived nitric oxide (Carlsson et al., 1987). More recently, several investigators have documented the importance of changes in transmembrane potential of vascular smooth muscle cells in the regulation of peripheral blood flow (Imamura et al., 1992; Samaha et al. 1992). Lombard et al. (1984) have proposed that by interfering with Ca^{2+} influx, changes in membrane potential lead to significant alterations in vascular smooth muscle tension. Among several ionic channels that regulate membrane potential of vascular smooth muscle cells, K_{ATP} channels have received considerable attention in the past several years. In addition to pancreatic β-cells, these channels have been identified in cardiac and skeletal muscles (Noma, 1983; Cooke and Hales, 1984). More recently, using compounds such as cromakalim and lemakalim which selectively activate K_{ATP} channels, several authors have demonstrated that these channels modulate in-vitro reactivity and in-vivo tone of various blood vessels and vascular beds in different animal species (Cavero et al., 1989; Quast and Cook, 1989; Hood et al., 1991; Jackson, 1993; Mayhan, 1993). In these experiments, glibenclamide selectively blocked the dilator responses mediated by activation of K_{ATP} channels. Moreover, Webb et al. (1989) reported that when cromakalim, a selective activator of K_{ATP} channels, was infused into the brachial artery of normal humans, forearm blood flow increased significantly with no effect on venous tone. These results suggest that K_{ATP} channels exist in

resistance arterioles of skeletal muscles and may play a role in the regulation of vascular tone in these muscles.

In our study, the decline in calf blood flow after glyburide intake can, therefore, be explained by the inhibition of K_{ATP} channels of vascular smooth muscle cells in the calf tissues. However, there are possible alternative mechanisms other than blocking smooth muscle K_{ATP} channels, through which glyburide may reduce baseline and reactive hyperemic calf flow. These include, firstly, a direct inhibition of mitochondrial function by glyburide which, in turn, causes inhibition of oxidative phosphorlyation and reduction in O_2 requirements and blood flow. However, Samaha et al. (1992) reported that myocardial mitochondrial respiration is not influenced by glyburide. Thus, reduction of blood flow by glyburide in our experiemnts is not likely to be due to inhibition of oxidative metabolism. Secondly, it is possible that glyburide reduces peripheral blood flow by directly stimulating vascular smooth muscle cells. We think that this is unlikely because glyburide elicits no significant changes in the vascular tone when K_{ATP} channels of vascular smooth muscle cells are inactive (Hu et al., 1990). Thirdly, a possible increase in circulating catecholamines as a result of glyburide-induced hypoglycaemia may contribute indirectly to the decline in peripheral blood flow in our experiments. However, baseline calf flow and post-occlusive flow were reduced at 1 h after glyburide intake. At this time, the changes in blood glucose were below those associated with a significant increase in catecholamine concentrations (Garber et al., 1976). Hence, the contribution of enhanced catecholamine release to the reduction in peripheral blood flow in our experiments is likely to be small. Finally, one may argue that glyburide reduces calf blood flow through the inhibition of endothelium-derived nitric oxide (NO). Although the importance of continuous NO release in the regulation of baseline blood flow in skeletal and cardiac muscles has been confirmed by several investigators (Gardiner et al., 1990; Hussain et al., 1992), it is not known whether glyburide affects basal NO release. Experimental evidence, however, indicates that K_{ATP} channels do not exist in the cell membrane of endothelial cells (Himmel et al., 1993). Accordingly, direct inhibition of endothelial cell NO production by glyburide is an unlikely cause of low calf blood flow.

4.3. Effects of glyburide on reactive hyperemia

In our study, a significant increase in post-occlusive calf flow was observed in response to a 10-min occlusion of the calf arterial supply. Skeletal muscle arteriolar dilation is known to develop after brief interruption of muscle blood flow. The mechanisms involved in this response are believed to be myogenic relaxation and the release of metabolic vasodilators (Carlsson et al., 1987); however, the exact contribution of these mechanisms to reactive hyperemia is still debatable. Myogenic relaxation which develops as a result of the decline in perfusion pressure during arterial occlusion, is likely to contribute to the immediate post-occlusive increase in blood flow, whereas metabolic dilation (including the direct effect of low P_{O_2} on smooth muscle of resistance vessels) may become involved in determining the duration of reactive hyperemia (Carlsson et al., 1987; Tuma et al., 1977). More recently, the vascular endothelium has also been implicated in the reactive hyperemic response of skeletal muscle (Ward et al., 1993).

In the present study, glyburide significantly attenuated the post-occlusive increase in calf blood flow in normal subjects. We propose that the effect of glyburide on post-occlusive flow is mediated through the inhibition of vascular smooth muscle K_{ATP} channels in the calf muscles. In a recent study, Daut et al. (1990) reported that glyburide infusion completely abolished the coronary dilation in response to arterial hypoxia and brief arterial occlusions in isolated guinea pig heart. These results indicate that smooth muscle K_{ATP} channels play an important role in the regulation of vascular tone in response to hypoxia and brief arterial occlusion. Although the exact mechanism through which these channels contribute to the vascular dilation during reactive hyperemia is not yet clear, it has been proposed that adenosine and ATP are involved (Nichols and Lederer, 1991). Adenosine release in response to arterial occlusion is likely to activate vascular smooth muscle K_{ATP} channels by binding to adenosine A_1 receptors. This eventually leads to membrane hyperpolarization and vascular relaxation. Alternatively, it is possible that arterial occlusion leads to a lowering of vascular smooth muscle capacity to generate ATP which, in turn, leads to removal of the inhibition of K_{ATP} channels and, therefore, to membrane hyperpolarization and vascular relaxation.

In summary, our study indicates that a single oral dose of glyburide elicits a significant decline in calf tissue blood flow and impairs reactive hyperemia of the calf tissue in normal subjects. Our results imply that therapeutic levels of glyburide may compromise peripheral blood flow regulation in patients with diabetes mellitus. We speculate that the increased incidence of cardiovascular complications seen in patients with oral glyburide may be due to blockade of K_{ATP} channels of vascular smooth muscle cells.

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