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The effect of glibenclamide on cutaneous laser-Doppler flux

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Abstract

The K_{ATP} channels play a crucial role in regulation of vascular tone in conditions of hypoxia. Whether they contribute to peripheral blood flow regulation in human cutaneous microcirculation during a non-hypoxic state is the matter of conflicting in vivo studies that have used plethysmographic method. Our aim was therefore to elucidate the role of K_{ATP} channels in human skin microcirculation in three different conditions that evoke different interplays of vascular mechanisms; during resting conditions, during the postocclusive vasodilatation and in the vasoconstriction response to local cold exposure. The laser-Doppler (LD) skin response was monitored in 12 healthy volunteers on the skin of the fingertips of both hands at rest, after the release of an 8-min digital arteries occlusion, and during local cooling of one hand at 15 °C. We compared the direct (at the measuring site) and the indirect (at the contralateral non-cooled hand) LD flux response after intradermal microinjection of saline solution (1 μl) and after a microinjection of the K_{ATP} channel blocker glibenclamide (8 μM saturated solution) at the measuring site after obtaining the dose-dependent effect of glibenclamide. The effect of the saline solution was used as a reference value. There was a statistically significant lower resting LD flux after the microinjection of glibenclamide 273.6±36 PU when compared to the values obtained after the application of the saline solution 375.8±31 PU (paired *t*-test, $p=0.016$). Glibenclamide also significantly reduced the relative area under the LD flux curve during the PRH response 14551±2508 PU*s vs. 6402±1476 PU*s (paired *t*-test, $p=0.01$) and increased the principal frequency of postocclusive PRH oscillations 0.0931±0.01 Hz vs. 0.1309±0.02 Hz ($p=0.01$). In addition, glibenclamide significantly decreased the LD flux during both the direct and indirect response to local cold exposure when compared to the application of saline solution (paired *t*-test, $p<0.01$). Our results support the conjecture that ATP sensitive K^+ channels are importantly involved in blood flow regulation of human skin microcirculation in PRH response, in resting conditions as well as in microvascular local cold response.

Keywords: Microcirculation; Skin; Laser-Doppler fluxmetry; Glibenclamide; K_{ATP} channels

Introduction

ATP-sensitive K^+ channels (K_{ATP}) have been identified in many types of cells and are involved in various physiologically important regulatory pathways (Quayle et al., 1997). They close as the intracellular concentration of ATP increases but are also regulated by mechanisms independent from ATP (Jackson, 2005).

K_{ATP} channels are also found in vascular smooth muscle cells of various vascular beds, including microcirculation (Nelson and Quayle, 1995; Tateishi and Faber, 1995; Jackson, 1993). Bari et al. have established the role of these channels in vascular tone regulation during hypoxia in piglets (Bari et al., 1998). Animal studies have also shown the regulatory role of K_{ATP} channels in response to the application of various drugs (Hammer et al., 2001; Garcia-Villalon et al., 1995; Jackson, 1993). The data from Jackson's study support the hypothesis that K_{ATP} channels also determine in part the resting arteriolar tone in animal skeletal muscle microcirculation in vivo (Jackson, 1993).

The experiments on human coronary arteries have provided evidence about the existence of K_{ATP} channels in human vascular smooth muscle cells (Gollasch et al., 1996). In spite of the extensive studies, the regulating role of these channels in various human vascular beds remains elusive. The majority of in vivo studies on human peripheral circulation have been preformed with the use of the noninvasive plethysmographic technique but the various authors have obtained controversial results regarding the role of K_{ATP} channels in regulation of human forearm blood flow. Some studies have showed an important role of K_{ATP} channels in regulation of resting forearm

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blood flow and during reactive hyperemia, induced by arterial occlusion (Kosmas et al., 1995; Bijlstra et al., 1996). The study of Banitt et al. also confirmed the contribution of these channels in post-occlusive hyperemia (PRH) (Banitt et al., 1996). Bank and associates reported the contribution K_{ATP} channels to ischemic vasodilation during PRH but not to resting blood flow regulation (Bank et al., 2000). In contrast, the study by Farouque and associates failed to show any effect of a K_{ATP} channel blockade on, PRH or an exercise-induced functional hyperemic response (Farouque et al., 2002; Farouque and Meredith, 2003a,b) but did confirm on the other hand, the important role of these channels in resting conditions. In view of these conflicting evidences, we attempted to clarify the regulating role of K_{ATP} channels with the use of alternative noninvasive method of peripheral blood flow measurements – laser Doppler (LD) fluxmetry. There are at list three advantages of this method. First, this technique allows us to measure the microvascular perfusion in a distinct part of human skin in contrast to combined skeletal muscle, adipose tissue and skin blood flow measured with plethysmographic method (Yvonne-Tee et al., 2006). Some studies indicate that hypoxia has a profound effect on muscle blood flow, potentially obscuring or exacerbating true chances in skin blood flow when using plethysmographic method (Dinenno, 2003; Halliwill, 2003). In contrast, LD fluxmetry allows one to measure changes in skin blood flow without measuring concomitant changes in underlying muscle (Minson, 2003). Second, our method excludes the possible effect of K_{ATP} channel blockade on conductive vessels that may interfere with the effect on microvasculature. There is substantial evidence from the pulmonary circulation for differences in expression and regulation of K^+ channels as one moves from conduit vessels toward the microcirculation (Archer et al., 1996; McCulloch et al., 2000). Third, our method allows us the use of topical application of minimum quantity of vasoactive drug with negligible systemic effects.

Our first objective was to determine whether K_{ATP} channels contribute to resting microvascular tone in skin of healthy humans. Apart from physiological implication, this question is particularly relevant in the situation of glibenclamide treatment of diabetic patients with compromised nutritive circulation of lower limb skin (Miura et al., 2003; Abbink et al., 2002). The second objective was to test hypothesis, that K_{ATP} channels are importantly involved in cutaneous PRH. The confirmation of this conjecture seems to be most likely, as the adenosine (activator), hypoxia and decreased perfusion pressure have been implicated in the mediation of reactive hyperemia in the condition of ischemia. (Hein and Kuo, 1999; Zhang et al., 2000; Chatterjee et al., 2003; Daut et al., 1990; Marshall et al., 1993). Some studies have also implicated the possible important contribution of K_{ATP} channels in some vasoconstrictive responses associated with adrenergic activity (Tateishi and Faber, 1995). Our third objective was therefore to evaluate the effect of K_{ATP} channel blockade during cutaneous response to local cooling. For all three experiment conditions, we evaluated the effect of specific K_{ATP} channel blocker glibenclamide on LD flux.

Subjects and methods

Subjects

Twelve young healthy volunteers (non-smokers, mean age 35±2.4), seven males and five females were recruited in the study. None of the subjects used any medication nor had a history of any diseases that involve even mild Raynaud's phenomenon. The National Ethics Committee approved the study, and informed consent was obtained from each subject.

Methods

The subjects were asked not to smoke or to drink coffee or tea for at least 8 h before the experiments. The measurements were performed at room temperature kept between 23 and 25 °C, 30 min after acclimatization. The subjects lay in a supine position and were instructed not to move during the measurements in order to avoid movement artifacts as much as possible.

LD flux

The LD flux was measured with two Periflux P4001 Master/4002 Satellite LD monitors (Perimed, Sweden). With this technique, the laser light is used to transluminate proximally one cubic millimeter of skin tissue and Doppler principle is adopted to measure the velocity of red blood cells in skin microvasculature. The LD flux signal is a stochastic representation of the number of moving cells in the tissue volume multiplied by their velocities. The output is referred to as perfusion flux that is expressed in perfusion units related to the Brownian motion in motility standard emulsion provided by the manufacturer. The principle governing the measurement of the skin perfusion with this technique has been described elsewhere (Nilsson, 1990; Yvonne-Tee et al., 2006). The LD probes (PF401) were attached to the pulp of one finger of both hands, and the measurements were done on both hands simultaneously. We refer to the LD flux changes obtained from the cooled hand as the direct response and LD flux changes from the contralateral hand as the indirect response. Power spectral analysis of LD flux was performed by the method of Fast Fourier Transform using Nevrokard software (Medistar, Slovenia).

Protocol

We measured the LD flux on a fingertip chosen randomly from the second, third or fourth finger for 6 min. The resting LD flux for each subject was determined as a 2-min average value.

The LD flux measurements were also performed for 6 min after the release of an 8-min digital arteries occlusion. For that purpose a miniature cuff was placed around the proximal phalange and inflated to 300 mm Hg. We calculated the following indices of the PRH response: the peak LD flux value during hyperemia, the time elapsed to the peak value, the time of PRH response and the area under the flux curve during the PRH response above resting values (relative area). We performed a Fast Fourier transform analysis of LD flux to obtain fundamental frequency of PRH oscillations defined as a component with the highest amplitude in 0.06–0.2 Hz band in the descending part of PRH curve (Strucl et al., 1994).

The LD flux was also monitored for 6 min before and for 6 min during the local cooling of one (always the left) hand. Local cooling was achieved with the flexible cold packs (Comfort Pack, 3M USA, 200×300 mm) at 15 °C; the hand was placed between two packs.

The measurements were repeated on the same subject after an intradermal microinjection of 1 μl of saline solution or after an injection of 1 μl 8 μM (saturated) solution of the specific K_{ATP} inhibitor glibenclamide at different randomly selected fingertips. The injections were made 1 mm from the measuring site. We obtained a dose–response curve to ascertain the specific effect and the amount of glibenclamide needed for the effect on the skin microcirculation (Fig 1). The measurements were performed at least 20 min after the injection to exclude the effect of injection trauma. LD flux values obtained after the injection of the saline solution were used as a reference value.

Statistics

The mean resting LD flux values after the application of glibenclamide and after the injection of the saline solution were compared by paired *t*-test.