Forearm endothelium-dependent vascular responses and the potassium-ATP channel

D. McAuley, C. McGurk, A. G. Nugent, C. Hanratty, S. Maguire & G. D. Johnston

The Department of Therapeutics and Pharmacology, The Queen's University of Belfast, Belfast BT9 7BL, Northern Ireland

Aims Vasodilation to acetylcholine is mediated at least in part by endothelium-derived hyperpolarising factor (EDHF) which causes membrane hyperpolarisation by activating potassium channels. It is however uncertain which potassium channel mediates this effect. The aim of this study was to determine the role of the potassium-ATP (K^+ -ATP) channel in mediating endothelium-dependent vascular responses to acetylcholine.

Methods In 10 healthy volunteers acetylcholine, an endothelium-dependent vasodilator, and sodium nitroprusside as a control assessing endothelium-independent vasodilatation were infused into the non-dominant brachial artery. Forearm blood flow (FBF) in response to each dose was measured by strain-gauge venous occlusion plethysmography. The K^+ -ATP channel blocker glipizide (2.5 mg) was then administered orally. After 45 min the infusions with FBF measurements were repeated.

Results Acetylcholine ($P < 0.01$) and sodium nitroprusside ($P < 0.01$) both caused an increase in FBF. There was no significant difference in vascular responses to acetylcholine ($P > 0.05$) or sodium nitroprusside ($P > 0.05$) following K^+ -ATP channel blockade.

Conclusions The K^+ -ATP channel does not modulate forearm arteriolar endothelium-dependent responses in healthy volunteers and therefore does not play a role in membrane hyperpolarisation.

Keywords: endothelium-dependent vasodilation, forearm blood flow, potassium-ATP channel, glipizide

Introduction

The vascular endothelium plays a central role in the regulation of vascular tone through the production of vasoactive substances, including the vasodilators nitric oxide and prostacyclin. Endothelium-derived hyperpolarising factor (EDHF) was identified following the recognition that endothelium-dependent vasodilation is not abolished by inhibition of both nitric oxide and prostacyclin [1].

EDHF activates potassium channels causing membrane hyperpolarisation. This inhibits voltage dependent calcium channels causing a reduction in intracellular calcium and vascular smooth muscle relaxation [2]. It is unclear which of several K^+ channels mediates this hyperpolarisation. The purpose of this study was to determine whether the K^+ -ATP channel modulates forearm endothelium-dependent responses to acetylcholine.

Methods

Ten healthy non-smoking volunteers (eight male and two female) of mean age 25.6 years (range 19–30) were studied. All abstained from alcohol and caffeine containing products

for 6 h and were given a standardised meal 1 h prior to the study. All gave written informed consent and approval was obtained from the local ethics committee. The studies took place in a temperature controlled room (24–26°C). Under local anaesthesia (1% lignocaine, Antigen Pharmaceuticals, Ireland) a 27-gauge needle was inserted into the non-dominant brachial artery. Blood flow at baseline and in response to each dose of drug infused was measured in both arms by strain-gauge venous occlusion plethysmography. An indium-gallium strain gauge coupled to an electronically calibrated plethysmograph (Medasonics model SPG16, California, USA) was connected to a two channel chart recorder (Medasonics model R12B) to record FBF. The mean of five consecutive measurements was taken for statistical evaluation.

Using a constant rate infuser (Braun Perfusor pump, Germany), acetylcholine (Iolab, UK) was infused into the experimental forearm in incremental doses of 60–240 nmol min⁻¹ for 3 min each, to assess endothelium-dependent vasodilatation. After a washout period basal FBF was again measured. Sodium nitroprusside (David Bull Laboratories, UK) was then infused in incremental doses of 3–12 nmol min⁻¹ to assess endothelium-independent vasodilatation. All infusions were at a rate of 1 ml min⁻¹. Glipizide (2.5 mg, Farmitalia Carlo Erba Ltd, UK) was then administered orally. After 45 min to allow absorption FBF was measured

Correspondence: Dr D. McAuley, The Department of Therapeutics and Pharmacology, The Queen's University of Belfast, The Whitla Medical Building, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland.

to re-establish baseline. The infusions of acetylcholine and sodium nitroprusside with FBF responses were then repeated.

Results are expressed as mean change in FBF from baseline ($100 \text{ ml}^{-1} \text{ min}^{-1}$) with 95% confidence intervals (CI) and were analysed using repeated measures analysis of variance. Basal blood flows were compared using a paired *t*-test.

The power to detect a change of 50% from mean values of FBF pre-glipizide at the 5% level of significance was 85%.

Results

FBF did not change significantly in the control arm during the period of the experiment confirming that drug effects were confined to the experimental forearm ($P > 0.05$).

Basal FBF did not differ significantly before and after glipizide for acetylcholine ($P > 0.05$) or sodium nitroprusside ($P > 0.05$). Acetylcholine ($P < 0.01$) and sodium nitroprusside ($P < 0.01$) both caused a dose dependent increase in FBF. There was no significant difference in forearm vascular response to either acetylcholine ($P > 0.05$) or sodium nitroprusside ($P > 0.05$) following glipizide.

Discussion

EDHF causing hyperpolarisation through K^+ channel activation, has been shown to account for 20–30% of the endothelium-dependent vasodilation seen in response to acetylcholine in the rat aorta [3]. We have demonstrated that K^+ -ATP channel blockade with the sulphonylurea, glipizide does not affect endothelium-dependent vascular responses suggesting that the K^+ -ATP channel is not involved in modulating this response.

As we have previously shown responses to these infusions are unaltered if are repeated on the same study day in the same subject without any intervention, it is unlikely that an order effect or tachyphylaxis occurred to confound these findings (unpublished data).

It is possible that glipizide or the small standardised meal (sandwich) could have had a significant haemodynamic action, causing increased FBF. As basal blood flow was unchanged before the sodium nitroprusside and acetylcholine infusions prior to the administration of glipizide (8.7; 95% CI 3.0–14.4 and 10.0; 95% CI 4.7–16.0 respectively), and before and after glipizide (8.7; 95% CI 3.0–14.4 and 6.0; 95% CI 3.3–8.7 respectively), this suggests that neither had a significant haemodynamic effect during the study. It is possible that insulin release may modulate endothelium-dependent responses. Although it has been shown that insulin does not effect endothelium-dependent vasodilation to acetylcholine *in vitro* in animal [4] and human [5] arteries,

Figure 1 Change in FBF from baseline in response to acetylcholine before (■) and after (▲) K^+ channel blockade (mean and 95% CI).

it has been shown to potentiate vasodilation to acetylcholine *in vivo* [6]. In view of this contradictory evidence it is difficult to conclude confidently that insulin did not modulate forearm vascular responses to acetylcholine.

Glipizide 2.5 mg reduces fasting blood glucose by 30–34% and increases immunoreactive insulin by about 100% in normal volunteers [7]. As insulin release is mediated through K^+ -ATP channel blockade in the beta islet cells this provides a biological assay confirming K^+ -ATP channel blockade. To prevent hypoglycaemia this study was carried out after a standard meal. As blood glucose and immunoreactive insulin will be necessarily higher after a meal and therefore not reflective of insulin release due to glipizide, measurement of these parameters was unlikely to have been helpful. Higher doses of sulphonylureas have been associated with blockade of K^+ -channels other than the ATP channel and were therefore avoided [8]. It should be pointed out that although peak absorption of glipizide occurs 1–3 h after oral administration, peak plasma concentration of insulin occurs after 30–60 min. As insulin release is the marker of K^+ -ATP channel blockade it is apparent this will be maximal at 30–60 min after oral administration and therefore the infusion of acetylcholine was repeated at this time [7, 9].

The finding that the K^+ -ATP channel is not involved in modulating endothelium-dependent vasodilation in response to acetylcholine is not entirely unexpected. The large conductance calcium-dependent potassium channels, rather than the K^+ -ATP channels, account for the large proportion of potassium ion transport across the cell membrane and are therefore more likely to be responsible for hyperpolarisation [10]. Further studies are required to determine the role of these calcium-dependent potassium channels.

Although our study involved healthy volunteers these

Table 1 Change in FBF from baseline in response to acetylcholine before and after K^+ channel blockade (mean and 95% CI).

Acetylcholine (nmol min^{-1})	Pre- K^+ -ATP blockade ($100 \text{ ml}^{-1} \text{ min}^{-1}$)	Post- K^+ -ATP blockade ($100 \text{ ml}^{-1} \text{ min}^{-1}$)	Difference pre/post K^+ -ATP blockade ($100 \text{ ml}^{-1} \text{ min}^{-1}$)
60	15.4 (7.7–23.1)	10.8 (3.7–17.9)	4.6 (0.0–9.1)
120	18.2 (7.2–29.2)	12.4 (4.1–20.8)	5.7 (–1.7–13.2)
180	18.4 (10.1–26.7)	16.9 (7.8–26.0)	1.5 (–4.2–7.2)
240	19.0 (10.4–27.7)	17.4 (8.0–26.8)	1.6 (–2.1–5.4)

findings have implications for the management of non-insulin dependent diabetes (NIDDM). As NIDDM is associated with impaired nitric oxide mediated endothelium-dependent vasodilation, hyperpolarisation may be of greater importance in the regulation of vascular tone. It is reassuring that K^+ -ATP channel blockade with glipizide, a sulphonylurea used widely to treat NIDDM, does not effect endothelial function.

In conclusion we have demonstrated *in vivo* that the K^+ -ATP channel does not modulate forearm arteriolar endothelium-dependent vascular responses to acetylcholine in healthy volunteers and therefore does not play a role in membrane hyperpolarisation.

References

- 1 Cohen RA, Vanhoutte PM. Endothelium dependent hyperpolarisation. Beyond nitric oxide and cyclic GMP. *Circulation* 1995; **92**: 3337–3349.
- 2 Quast U. Do the K channel openers relax smooth muscle by opening K channels? *Trends Pharmacol Sci* 1993; **14**: 332–337.
- 3 Van de Voorde J, Vanheel B, Leusen I. Endothelium dependent relaxation and hyperpolarisation in aorta from control and renal hypertensive rats. *Circulatory Res* 1992; **70**: 1–8.
- 4 Turner NC, Gudgeon C, Toseland N. Effects of genetic hyperinsulinaemia on vascular reactivity, blood pressure, and renal structure in the Zucker rat. *J Cardiovasc Pharmacol* 1995; **26**: 714–720.
- 5 McNally PG, Lawrence IG, Watt PAC, Hillier C, Burden AC, Thurston H. The effect of insulin on the vascular reactivity of isolated resistance arteries taken from healthy volunteers. *Diabetologia* 1995; **38**: 467–473.
- 6 Taddei S, Virdis A, Mattei P, Natali A, Ferrannini E, Salvetti A. Effect of insulin on acetylcholine-induced vasodilation in normotensive subjects and patients with essential hypertension. *Circulation* 1995; **92**: 2911–2918.
- 7 Pedrazzi F, Ceretti AP, Losi S, Bommartini F, Artini D, Amaneuli A. Evaluation in hospitalised subjects of a new hypoglycaemic sulphonylurea, glydiazinamide. *Arzneimittel-Forschung* 1971; **21**: 222.
- 8 Gelband CH, McCullough JR. Modulation of the rabbit aortic Ca activated K channels by pinacidil, cromakalim, and glibenclamide. *Am J Physiol* 1993; **264**: C1119–27.
- 9 Balant L, Fabre J, Zahnd G. Comparison of the pharmacokinetics of glipizide and glibenclamide in man. *Eur J Clin Pharmacol* 1975; **8**: 63–68.
- 10 Nelson MT, Patlak JB, Worley JF, Standen NB. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am Physiol Soc* 1990; **90**: C3–C18.

(Received 15 August 1996,
accepted 16 May 1997)