Placenta (2006), **27**, 660–668
doi:10.1016/j.placenta.2005.05.012

Glibenclamide Inhibits Agonist-induced Vasoconstriction of Placental Chorionic Plate Arteries

M. Wareing*, S. L. Greenwood, G. K. Fyfe, P. N. Baker and M. J. Taggart

Maternal and Fetal Health Research Centre, University of Manchester, St. Mary's Hospital, Hathersage Road, Manchester, M13 0JH, UK

Paper accepted 16 May 2005

Background: Preliminary data suggest that K_{ATP} channels may be expressed in placental arteries and veins [Wareing M, Turner C, Greenwood SL, Baker PN, Fyfe GK. Expression of mRNA encoding K^+ channels in chorionic plate arteries and veins. J Soc Gynecol Investig 2004;11:353A]. However, no data exist on glibenclamide's effects in placental chorionic plate arteries.

Aim: To assess the effect of glibenclamide on placental chorionic plate arterial vasoconstriction.

Methods: Arteries were dissected from placental chorionic plate biopsies obtained at term from uncomplicated pregnancies ($N = 63$). Arteries were mounted onto a wire myograph in HCO_3^--buffered physiological salt solution (PSS) at 37 °C (5% O_2/5% CO_2 bubbling) and normalised at 0.9 of $L_{5.1\,kPa}$. Constriction viability was assessed with 120 mmol l^{-1} potassium solution (KPSS). Dose–response curves were produced with the thromboxane-mimetics U46619 and U44069 (10^{-10}–2×10^{-6} M), arginine vasopressin (10^{-10}–5×10^{-8} M) and endothelin-1 (10^{-11}–3×10^{-7} M) in the presence or absence of 50 μmol l^{-1} glibenclamide. The effect of glibenclamide on arginine vasopressin- and U46619-induced constriction was also assessed in the presence of the cyclo-oxygenase inhibitor indomethacin (10 μmol l^{-1}).

Results: Pre-incubation with 50 μmol l^{-1} glibenclamide significantly right-shifted dose–response curves to all vasoconstrictive agonists tested (repeated measures ANOVA). Indomethacin did not modify the inhibitory effect of glibenclamide.

Conclusion: Glibenclamide's effects on agonist-induced constrictions are unlikely to be via an inhibition of ATP-sensitive K^+ channels, and with U46619- and U44069-induced constrictions, glibenclamide may be acting as a competitive antagonist of thromboxane receptors.

Placenta (2006), **27**, 660–668

Keywords: Placenta; Artery; Human; Potassium channels

INTRODUCTION

The completion of a successful pregnancy requires an adequately perfused fetoplacental vasculature, yet the mechanisms responsible for the control of vasomotor tone and blood flow within the fetoplacental circulation are poorly understood.

K_{ATP} channels have been identified pharmacologically and molecularly in a range of cell types including vascular smooth muscle cells and pancreatic β cells [1–4]. In systemic vascular smooth muscle cells, activation of these channels promotes membrane hyperpolarisation, closure of voltage-gated calcium channels and subsequent smooth muscle relaxation; this mechanism is exploited, at least in part, by a range of pharmacological vasodilatory agents such as pinacidil, nicorandil and cromakalim [1,5,6]. Such agents have been used as pulmonary vasodilators to alleviate inappropriate vasoconstriction caused by, for example, hypoxia [2].

Other pharmacological agents that target K_{ATP} channels include glibenclamide, a sulfonylurea, that has been used clinically to treat type-2 diabetes via an inhibitory action on pancreatic β cell K_{ATP} channels [1,7–9]. At micromolar concentrations, glibenclamide has been shown to block K_{ATP} channels in a variety of vascular smooth muscle preparations [10–13] and in intact blood vessels [14,15].

The presence of K_{ATP} channels in fetoplacental blood vessels may be important for the regulation of normal fetoplacental blood flow. As fetoplacental vessels are not innervated [16], the control of vascular tone must be mediated at a local level. Thus, activation of K_{ATP} channels may offer such a local mechanism to control vascular smooth muscle excitability and contractility and thus modify flow through placental blood vessels. Modification of K_{ATP} channel activity may also offer a possible therapeutic target for pregnancy complications such as intrauterine growth restriction (IUGR),

* Corresponding author. Tel.: +44 161 276 5474; fax: +44 161 276 6134.
E-mail address: mark.wareing@manchester.ac.uk (M. Wareing).

0143–4004/$–see front matter

where data suggest an inappropriately constricted vasculature, in concert with altered vascular morphology, may lead to pathology [17,18].

Preliminary studies in our laboratory [19] have suggested the presence of mRNA for $K_{IR}6.1$, the pore-forming subunit of an ATP-sensitive K^+ channel [20] in placental arteries and veins. However, only a small number of studies have assessed a possible role for K_{ATP} channels in the human placental arteries [21–23]. Guiet-Bara et al. have suggested the presence of K_{ATP} channels by alteration of membrane potential following addition of glibenclamide to vascular smooth muscle cells from human allantochorial placental vessels; conversely they were unable to detect similar changes in endothelial cells [21,22]. In contrast, using the whole cell patch clamp technique in smooth muscle cells isolated from "small fetoplacental arteries", Hampl et al. [23] were unable to detect ATP-dependent currents. Therefore, the role of K_{ATP} channels in the control of fetoplacental vascular function remains unresolved and the effect of glibenclamide on placental vascular tone is untested.

Therefore, the aim of the current study was to assess whether K_{ATP} channels are functionally present in human chorionic plate arteries by performing a wire myography study, with a range of vasoconstrictors (U46619, U44069, arginine vasopressin, endothelin-1), and assess the influence of glibenclamide, a blocker of K_{ATP} channels and pinacidil, an opener of K_{ATP} channels.

MATERIALS AND METHODS

This work was performed with the approval of the ethics committee of Central Manchester Healthcare Trust. Informed written consent was obtained for all tissue used in the study.

Samples

Term (37–42 weeks gestation) placentae ($N = 63$) were obtained post-delivery (vaginal or after elective Caesarean section) from women with otherwise uncomplicated pregnancies (no evidence of hypertension, intrauterine growth restriction or other medical disorders). Biopsies were taken within 20 min of delivery and placed directly into ice-cold physiologic salt solution (PSS; in mmol l^{-1}; 127.76NaCl, 25$NaHCO_3$, 4.69KCl, 2.4$MgSO_4$, 1.6$CaCl_2$, 1.18KH_2PO_4, 6.05 glucose, 0.034 EDTA; pH 7.4). Small arteries were dissected under a stereomicroscope, cut into 2–3 mm lengths and mounted onto M610 wire myograph (Danish Myotech, Aarhus, Denmark).

Myography

Chorionic plate arteries were mounted onto 40 μm steel wires, bathed in 6 ml of PSS and warmed to 37 °C. The baths were gassed with 5% CO_2 in 5% O_2 (BOC special gases, Worsley, UK) to give a final dissolved oxygen content of 4.5–5.8% (termed 7%; 52 mmHg) measured using a WPI oxygen metre (WPI Inc., USA; measurement accuracy ± 1%). Chorionic plate arteries were normalised to 0.9 of $L_{5.1\ kPa}$ to mimic a physiological resting tension of approximately 25 mmHg [24]. Post-normalisation arteries were equilibrated for 20 min prior to the commencement of vasoactive studies. Vessels greater than 500 μm in diameter were excluded from the study.

Baseline (passive) tension

The effects of pinacidil (50 μmol l^{-1}) and glibenclamide (50 μmol l^{-1}) were assessed on unstimulated placental vessels. Basal tone was assessed pre- and 5 min post-addition of the agent to the bath.

Agonist-induced vasoconstriction

Placental vessel viability was assessed with two successive exposures to 120 mmol l^{-1} KCl in PSS (KPSS; equimolar substitution of KCl for NaCl). Vasoconstriction of chorionic plate arteries was assessed with the thromboxane-mimetics U46619 and U44069 (both 10^{-10}–2×10^{-6} M), arginine vasopressin (AVP; 10^{-10}–5×10^{-8} M) and endothelin-1 (ET1; 10^{-11}–3×10^{-7} M). Experiments were performed in parallel in the presence of 1–50 μmol l^{-1} glibenclamide (10 mmol l^{-1} stock dissolved in 5:1 DMSO:ethanol v/v). Glibenclamide was added 5 min prior to commencement of the agonist dose–response curve. Time control vessels received an equal volume of vehicle.

In a sub-group of vessels constricted with 1 μmol l^{-1} U46619, pinacidil (50 μmol l^{-1}) was added at peak constriction. Two minutes post-pinacidil addition, 50 μmol l^{-1} glibenclamide or an equal volume of vehicle (as a time control) was added to the bath.

Role of cyclo-oxygenase in arterial response to agonist stimulation

Experiments with U46619-, ET1- and AVP-induced constrictions were performed as described above, except that post-normalisation, chorionic plate arteries were exposed to the cyclo-oxygenase inhibitor indomethacin (10 μmol l^{-1}) 20 min prior to commencement of the agonist-induced vasoconstriction protocols. Indomethacin was present for the duration of the experimental protocol.

General chemicals

General chemicals and pharmacological agents were obtained from either Sigma-Aldrich (Poole, Dorset, UK) or BDH (Poole, Dorset, UK). U46619 and glibenclamide were obtained from Calbiochem (CN Biosciences (UK) Ltd., Nottingham, UK).

Statistical analysis

Vessel tension production was expressed as P_i (active effective pressure) in kPa. P_i is calculated from the active wall tension ΔT (mN/mm) divided by the normalised internal radius (mm) of the vessel. Data for the effect of glibenclamide and pinacidil on baseline (passive) resting tension were compared using