Indiscriminative Effects of Repaglinide and Other Specific Modulators of Transmembrane KATP-Channel Gating Properties upon Ischaemic/Hypoxic Bovine Coronary Artery Smooth Muscle Relaxation

Moosbrugger B, Salzer M

Homepage:
www.kup.at/jcbc
Online Data Base Search for Authors and Keywords
Indiscriminative Effects of Repaglinide and Other Specific Modulators of Transmembrane $K_{\text{ATP}}$-Channel Gating Properties upon Ischaemic/Hypoxic Bovine Coronary Artery Smooth Muscle Relaxation

R. Gasser$^1$, H. Köppel$^2$, B. Moosbrugger$^1$, G. Esche$^1$, M. Salzer$^1$, S. Gasser$^1$, S. Holzmann$^3$

The opening of $K_{\text{ATP}}$ channels both in myocardium and vascular smooth muscle constitutes an important mechanism in the regulation of myocardial metabolism and perfusion, in particular, during hypoxia and ischaemia. In coronary smooth muscle, $K_{\text{ATP}}$ channel opening invariably leads to vasodilation and hence to improved myocardial perfusion. Blockade of these channels may disturb this pivotal response. We compare the effects of glibenclamide with two new sulfonylureas on coronary artery dilation secondary to hypoxia and simulated ischaemia. We use bovine coronary arteries in a modified Tyrode solution (equilibrated with 95 % O$_2$ and 5 % CO$_2$ at 37 °C at a constant pH of 7.4). The solution contained the following solutes (in mM): NaCl 112.8, KCl 26.8, CaCl$_2$ 1.36, MgSO$_4$ 1.16, NaHCO$_3$ 11.9, glucose 10.1. Hypoxia was mimicked by changing 95 % O$_2$ to 95 % N$_2$ in the perfusate. Ischaemia was simulated biochemically using iodoacetate (IAA; 0.5 mM) and dimetiron (DNP; 1 mM).

We found competitive binding of the three drugs (glibenclamide, glimepiride and repaglinide) to $K_{\text{ATP}}$ channels with pinacidil and cromakalim showing similar dose response curves. Hypoxic control arteries relaxed by 50 %, which was invariably reduced by the three drugs in a dose dependent manner (for glibenclamide and repaglinide to 16 %, by glimepiride to 20 %). Similar results were obtained for DNP IAA, an inhibitor of G3P-dehydrogenase, relaxed the arteries, but relaxation could not be affected by either drug (effects of glycolytic metabolites on $K_{\text{ATP}}$ channel modulation).

Our results show that newly designed sulfonylureas have the same effects on coronary arteries as the well known old drug glibenclamide. The experiments provide insight into one particular aspect of the cardiovascular effects of these drugs, namely on hypoxic vasodilation. Since UKPDS provides clinical evidence for the cardiovascular safety of glibenclamide, the coronary effects of sulfonylureas may not be of major importance as far as the hard endpoints of the study are concerned. Possibly $K_{\text{ATP}}$ channel opening exerts clinically relevant effects more in the sense of transient regulation of metabolism and perfusion than by myocardial protection in the case of myocardial infarction itself and other major cardiovascular events. J Clin Basic Cardiol 2003; 6: 81–5.

**Key words:** repaglinide, glimepiride, ischaemia, hypoxia, coronary artery

The opening of $K_{\text{ATP}}$ channels during myocardial ischaemia has various important cardioprotective functions [1]: it is involved in ischaemic preconditioning [2–5], shortening of the action potential duration (decreased calcium influx) [6, 7] and mediates ischaemia induced K-efflux [7, 8]. In arteriolar smooth muscle, the opening of $K_{\text{ATP}}$ channels invariably leads to vasodilation [9–11]. In particular, during ischaemia, this dilation of the coronary arteries constitutes a pivotal mechanism designed to increase perfusion in the ischaemic tissue [9, 12]. Inhibition of $K_{\text{ATP}}$ channel opening by specific blockers, like sulfonylureas, may be harmful under certain conditions [13–15]. Therefore, the potential danger of oral antidiabetics in patients with coronary heart disease has been discussed for many years. The discussion was sparked off by the disclosure of data from a large clinical trial, UGDP: it evidenced that the sulfonylurea compound tolbutamide increased cardiovascular mortality versus placebo in type II diabetes – an observation not readily explained as yet [14]. These data are now contradicted by another large trial, UKPDS, which showed no significant difference in cardiovascular mortality in patients with and without sulfonylureas [16]. While, on the one hand, $K_{\text{ATP}}$ channel blockade by sulfonylureas was looked at as beneficial, in particular as far as arrhythmias are concerned [7, 17–19] on the other hand, inhibition of preconditioning [4, 20] and hypoxic vasodilation [13, 20] has to be viewed with caution. The cardiovascular safety of glimepiride has been used as an important argument [21–24]. For repaglinide [25], a comparable new compound, cardiovascular effects have not been looked at so far.

Here we compare the pharmacological actions of glibenclamide, glimepiride and repaglinide upon coronary artery smooth muscle during hypoxia and simulated ischaemia (iodoacetate [IAA], dimetiron [DNP]). We show that there is no difference in effect on coronary artery dilation secondary to hypoxia and simulated ischaemia. Our data suggest that neither of the drugs has favourable effects on hypoxic/ischaemic coronary artery dilation.

**Material and Methods**

**Mechanical Experiments**

Circular strips of coronary arteries (1.5–2 mm diameter) were taken from bovine hearts and carefully freed from adherent connective tissue. All procedures were performed carefully so that the endothelium remained intact. The hearts were collected from a local abattoir and brought to our laboratory in virtually ice-cold Tyrode's solution. Blood was rinsed from the lumen and sixteen circular strips were then suspended simultaneously in organ baths of 5 ml volume (Hugo Sachs, each in a modified Tyrode's solution). Isotonic changes in length were measured by a mechano-electronic transducer (Hugo Sachs) and continuously recorded on a Watanabe

Received: August 18th, 2003; accepted September 26th, 2003.
From the $^1$Department of Cardiology, the $^2$Department of Angiology and the $^3$Department of Pharmacology and Toxicology, University of Graz, Austria

Correspondence to: Prof. Robert Gasser, MD, PhD, Department of Cardiology, Medizinische Universitätsklinik Graz, Auenbruggerplatz 15, A-8036 Graz, Austria; e-mail: robert.gasser@uni-graz.at

For personal use only. Not to be reproduced without permission of Krause & Pachernegg GmbH.
multirecorders. The strips stabilised in length within 90 minutes incubation with 26.8 mM K+ at about 60 % of the maximal shortening which was determined repeatedly by the addition of 80 mM (= 30-fold) K+ and remained constant throughout the experiments. Relaxation was obtained at the end of each experiment by addition of 2.66 × 10−4 M papaverine and related to the control length before the addition of the drugs. The method used for our experiments has been used successfully in our laboratories since 1970 and further details can be retrieved from earlier publications [26–28].

Solutions
In all experiments we used CO2-bicarbonate buffered modified Tyrode’s solution. Solutions were equilibrated with 95 % O2 and 5 % CO2 at 37 °C at a constant pH of 7.4. The solution contained the following solutes (in mM): NaCl 112.8, KCl 26.8, CaCl2 1.36, MgSO4 1.16, NaHCO3 11.9, glucose 10.1. Solutions were prewarmed in a water jacketed vessel to the desired temperature of 37 °C. Hypoxia was brought about by switching 95 % O2 and 5 % CO2 to 95 % N2 and 5 % CO2 in the solutions. Glibenclamide (Hoechst, Germany), glimepiride and repaglinide (both received as a gift from Novo Nordisk Austria) were dissolved in dimethylsulfoxide (DMSO, Fluka Switzerland) and added to the solution, so that the final concentration did not exceed 0.3 % of the total volume of the Tyrode’s solution. Iodoacetate (IAA, Sigma), an SH-group containing inhibitor of glycolytic key enzymes was added to the solutions in a concentration of 0.5 mM. Dinitrophenol (DNP) was added in a concentration of 1 mM. These drugs are commonly used to simulate ischaemia [29–31]. Both cromakalim and pinacidil were used at various concentrations and also dissolved in DMSO.

Results
Like glibenclamide, glimepiride and repaglinide show a weak intrinsic relaxing activity culminating at concentrations of 10−5 M at 20 and 36 % respectively (Fig. 1a, b). Repaglinide begins to relax bovine coronary artery strips at a concentra-

![Figure 1](https://example.com/fig1.png)

**Figure 1.** A: Intrinsic relaxing activity of repaglinide in bovine coronary artery in percent of maximal contraction (obtained at the end of each experiment by the addition of 2.66 × 10−4 M papaverine). Strips stabilised in length within 90 min of incubation at about 60 % of maximal shortening; B: Intrinsic relaxing activity of glimepiride at different concentrations like in 1A

![Figure 2](https://example.com/fig2.png)

**Figure 2.** Experiment like in Fig. 1A; repaglinide shows competitive inhibition of specific K channel openers (here shown: pinacidil)

![Figure 3](https://example.com/fig3.png)

**Figure 3.** A: Relaxation of bovine coronary arteries during hypoxia (open symbols) and its reversibility by reoxygenation; glibenclamide (2 µM) inhibits hypoxic vasodilation; glibenclamide’s intrinsic relaxing activity can be seen in the reoxygenation curve in form of a slight diminution of recovery; B: Dose dependent inhibition by repaglinide of hypoxic vasodilation; C: Dose dependent inhibition by glimepiride of hypoxic vasodilation. The intrinsic relaxing activity of glimepiride can be seen in form of a 17 % initial relaxation of the arteries. This effect was not so pronounced with repaglinide (B)
Iodoacetate (IAA) inhibits the glyceraldehyde-3-phosphate dehydrogenase activity of 3-phosphoglycerate kinase. Glimepiride is an even weaker vasorelaxant. At concentrations of 3 \times 10^{-7} M a relaxation of 4 \pm 0.5 % is seen and shows its maximal effect at 10^{-5} M with 19 \pm 1.6 % (Fig. 1b).

**Dose-Response Curves with Cromakalim and Pinacidil**

We found that both repaglinide and glimepiride antagonise the K<sub>ATP</sub> channel openers cromakalim and pinacidil and shift the relaxation curves in a dose-dependent manner to the right. Figure 2 shows the relaxation and dose response curves of repaglinide with cromakalim and pinacidil at concentrations of 3 \times 10^{-8}, 3 \times 10^{-7}, 1 \times 10^{-6} and 3 \times 10^{-6} M repaglinide as an example (ED 50 = 1.4 \times 10^{-6}, n = 6; K<sub>B</sub> = 4.06 \pm 0.67 \times 10^{-8} M and p<sub>A2</sub> = 7.4 \pm 0.73). These values are in accordance with the values of the Schild-blot and therefore repaglinide shows competitive inhibition of specific K channel openers. Similar results were obtained for glimepiride.

**Hypoxia**

As put forward in the Method section, hypoxia was simulated by switching 95 % O<sub>2</sub> and 5 % CO<sub>2</sub> to 95 % N<sub>2</sub> and 5 % CO<sub>2</sub> in the perfusate. Within 60 minutes, control arteries relaxed by approximately 50 % (Fig. 3a). Hypoxic vasorelaxation could be reversed by reoxygenation. As a reference substance 2 \mu M glibenclamide reduced maximal hypoxic vasorelaxation to 16 \pm 3.33 % (Fig. 3a). This confirms earlier findings of other authors and ourselves which show that K<sub>ATP</sub> channel opening is involved in hypoxic vasodilatation. Repaglinide, however, showed a similar effect on hypoxia-induced vasorelaxation in a dose-dependent manner, beginning at 3 \times 10^{-6} M (Fig. 3b). Similar results could be produced by glimepiride. Concentrations of 10^{-7}, 10^{-9} and 3 \times 10^{-9} M glimepiride reduced hypoxic vasorelaxation from a maximum of 41.5 \pm 5.12 to 28.3 \pm 4.1, 29.5 \pm 2.8 and 20.3 \pm 2.3 % respectively (Fig. 3c). Upon reoxygenation, the described phenomena could be reversed. In some experiments, the smooth muscle tone was slightly increased after reoxygenation.

**Simulated Ischaemia**

Ischaemia was simulated by pharmacological manoeuvres which are known to interfere with cellular energy metabolism or delivery.

- Dinitrophenol (DNP), an inhibitor of oxidative phosphorylation led to vasodilation up to 60 %. This is comparable with the vasorelaxation seen under hypoxia. Repaglinide showed a dose-dependent inhibition of relaxation. The highest concentration of repaglinide inhibited DNP-induced vasorelaxation to the same extent as 2 \times 10^{-6} M glibenclamide (Fig. 4a, b). Glimepiride equally showed a dose-dependent inhibition and 3 \times 10^{-6} M glimepiride showed an inhibition of DNP-induced vasorelaxation equal to 2 \mu M glibenclamide (Fig. 4c). Inhibition of DNP induced vasorelaxation is slightly less pronounced with repaglinide (Fig. 4b).

- Iodoacetate (IAA) inhibits the glyceraldehyde-3-phosphate dehydrogenase, thus constituting an inhibitor of glycolysis. 5 \times 10^{-4} M iodoacetate led to a relaxation of the arteries which could not be attenuated by either glibenclamide, repaglinide or glimepiride (Fig. 5; see Discussion).

**Discussion**

The ATP dependent potassium channel, which has been known for a long time as the essential mediator for insulin release from the pancreatic beta-cells, has also been described in various other tissues including the myocardium and arterial smooth muscle. In myocardium, Noma demonstrated this inwardly rectifying K current as I<sub>ATP</sub> in 1983 [32]. Its function in the context of hypoxia and ischaemia was revealed in the late 1980s, both in ventricular myocardium and arterial smooth muscle [1, 11]. During recent years, the cardioprotective role of K<sub>ATP</sub> channel opening during ischaemia has received major attention [2–4, 33, 34]. Several effects of K<sub>ATP</sub> channel opening were put forward as favourable during
ischaemia: The shortening of the plateau phase of the cardiac action potential (AP) with its inherent diminution of net calcium influx may locally reduce contractility and thus oxygen consumption of the ischaemic tissue. This AP-shortening could also constitute the first step to hibernation with calcium as an intracellular second messenger inducing the molecular adaptation for the long term decrease in contractility [35]. Preconditioning, another well known cardioprotective phenomenon in repetitive ischaemia, is similarly brought about by $K_{ATP}$ channel opening. Undoubtedly, coronary vasodilation during hypoxia and ischaemia constitutes a physiological mechanism designed to improve coronary blood flow to the ischaemic tissue [9, 10, 12, 13, 15, 28].

The pending discussion on cardiovascular safety of sulfonylureas, which was started in the 1970s by the results of UGDP has been nourished by the findings related to cardioprotection by $K_{ATP}$ channel opening. UGDP assessed the efficacy of tolbutamide (a specific $K_{ATP}$ channel blocker) in comparison to insulin and diet alone in the prevention of cardiovascular complications in NIDDM patients [14]. The surprising finding of a significantly higher cardiovascular mortality in patients treated with sulfonylureas compared to placebo eventually found its explanation in the myocardial $K_{ATP}$ channel. The call for reconsideration of sulfonylurea treatment as well as for designing new sulfonylureas hence became louder. Last year’s release of the UKPDS data inflamed the controversy on possible side effects of sulfonylureas de novo, since UKPDS showed no significantly increased cardiovascular mortality through the use of glibenclamide. While the discussion lingers on, the possible cardioprotective effects of $K_{ATP}$ channel opening gave rise to a large mortality study using the $K_{ATP}$ channel opener nicorandil in patients with coronary heart disease [36]. Our data compare the effects of 3 different sulfonylureas on coronary artery dilation secondary to hypoxia and simulated ischaemia.

Before actually discussing our findings, we have to consider the validity and limitations of our technique. By definition, “ischaemia” can only be brought about in blood-perfused organs. This can solely be achieved in in vivo situations and will always be inappropriate in vitro. An established method to simulate an ischaemia-like condition, however, is the generation of hypoxia/anoxia by switching oxygen to nitrogen in the perfusate. Another method to simulate ischaemia is by mimicking its salient characteristic, a disturbance in cellular energy balance. Here we have used two established methods, glycolytic inhibition by IAA (a blocker of glyceraldehyde-3-phosphate-dehydrogenase) and DNP (an uncoupler of oxidative phosphorylation). In a recent paper we have, however, shown that inhibiting cellular energy production or transmission at different stages of cellular metabolism elicits differing responses of arterial relaxation and leads similarly to differing responses to $K_{ATP}$ channel blockade. Therefore, we used 3 different methods to simulate hypoxia/ischaemia. The mechanical experiments on bovine coronary arteries in perfusion baths have been performed successfully in our laboratory since 1970, the validity of these experiments was discussed earlier [7].

**Dose-Response Curves**

The 3 different drugs, glibenclamide, glimepiride and repaglinide show similar dose-response curves to $K_{ATP}$ channel openers like cromakalim or pinacidil with comparable ED$_{50}$, $K_{B}$ and pA$_{2}$ values. These results confirm that all 3 drugs are specific blockers of $K_{ATP}$ channels in vascular smooth muscle cells.

**Hypoxia**

Changing oxygen to nitrogen in the perfusate invariably led to vasorelaxation, the latter could be augmented by equimolar doses of the 3 drugs. There was no statistically significant difference, nor was there a lack of inhibition with any of the drugs. We conclude that neither of the newly designed sulfonylureas is different with respect to the response to hypoxic vasodilation.

**Simulated Ischaemia**

The relaxation brought about by DNP was invariably inhibited by the 3 drugs as can be seen in Figure 4a–c. However, in the presence of IAA, none of the drugs was able to inhibit vasodilation significantly (Figure 5a–c). These findings are in accordance with our earlier publication, which shows that certain glycolytic products are necessary for the blockade of $K_{ATP}$ channels by sulfonylureas which could result from
References


4. Engler RL, Yellon DM. Sulfonylurea K ATP blockade in type II diabetes and the results of cardiovascular effects of these drugs, namely on hypoxic vasodilation. They show that in this context, neither of the drugs is preferential to glibenclamide. The recent results of UKPDS provide clinical evidence for the safe use of sulfonylureas in NIDDM, as far as cardiovascular events are concerned. Possibly, K ATP channel opening exerts clinically relevant effects more in the sense of transient regulation of metabolism and perfusion than by effective myocardial protection in the case of myocardial infarction itself.

In summary, our data show that newly designed sulfonylureas have the same effects on coronary arteries as the well known old drug glibenclamide. The experiments presented support the hypothesis that there is a lack of cardiovascular effects of these drugs, namely on hypoxic vasodilation. They show that in this context, neither of the drugs is preferential to glibenclamide. The recent results of UKPDS provide clinical evidence for the safe use of sulfonylureas in NIDDM, as far as cardiovascular events are concerned. Possibly, K ATP channel opening exerts clinically relevant effects more in the sense of transient regulation of metabolism and perfusion than by effective myocardial protection in the case of myocardial infarction itself.

