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ORIGINAL PAPERS, BASIC CARDIOLOGY

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

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The opening of K_{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_{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 dinitrophenol (DNP; 1 mM).

We found competitive binding of the three drugs (glibenclamide, glimepiride and repaglinide) to K_{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_{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_{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_{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 arterial smooth muscle, the opening of K_{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_{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 diabetics – 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_{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 (iodoacetic acid [IAA], dinitrophenol [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 4 Watanabe

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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 experiment. 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 CO_2-bicarbonate buffered modified Tyrode's solution. Solutions were 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. Solutions were prewarmed in a water jacketed vessel to the desired temperature of 37 °C. Hypoxia was brought about by switching 95 % O_2 and 5 % CO_2 to 95 % N_2 and 5 % CO_2 in the solutions. Glibenclamide (Hoechst, Germany), glimepiride and repaglinide (both received as a gift from Novo Nordisk Austria) were dissolved in dimethylsulfoxyde (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-

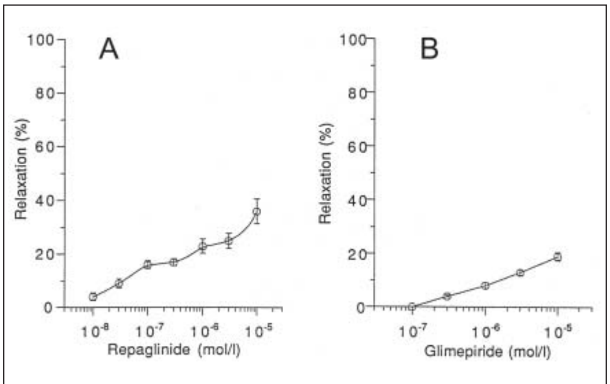

Figure 1. A: Intrinsic relaxing activity of repaglinide in bovine coronary artery in percent of maximal contraction (obtained at the end of each experiment by the addition of 2.66×10^{-4} M papaverine). Strips stabilised in length within 90 min of incubation at about 60 % of maximal shortening; **B:**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

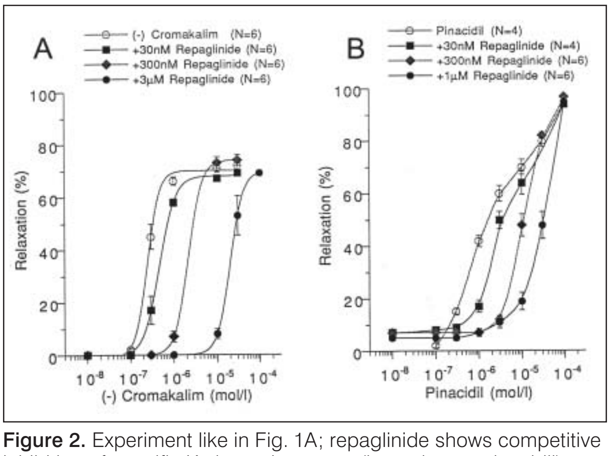

Figure 2. Experiment like in Fig. 1A; repaglinide shows competitive inhibition of specific K channel openers (here shown: pinacidil)

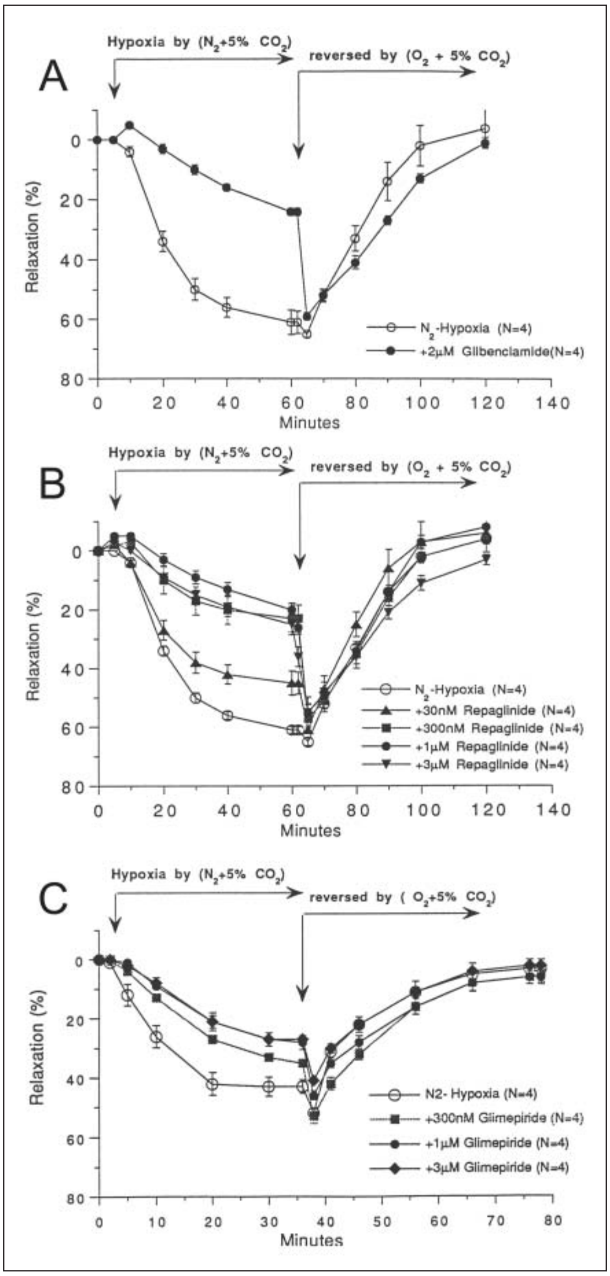

Figure 3. A: Relaxation of bovine coronary arteries during hypoxia (open symbols) and its reversibility by reoxygenation; glibenclamide (2 µM) inhibits hypoxic vasodilation; glibenclamide's intrinsic relaxing activity can be seen in the reoxygenation curve in form of a slight diminution of recovery; **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)**

tion of 10^{-8} M with 4 ± 1.5 % (n = 4; ± SEM) and at 10^{-5} M with 36 ± 4.6 % (n = 4, ± SEM). The latter constitutes the maximal possible relaxation inducible by the drug (Fig. 1a). Glimepiride is an even weaker vasorelaxant. At concentrations of 3×10^{-7} M a relaxation of 4 ± 0.5 % is seen and shows its maximal effect at 10^{-5} M with 19 ± 1.6 % (Fig. 1b).

Dose-Response Curves with Cromakalim and Pinacidil
We found that both repaglinide and glimepiride antagonise the K_{ATP} 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 3×10^{-8}, 3×10^{-7}, 1×10^{-6} and 3×10^{-6} M repaglinide as an example (ED 50 = 1.4×10^{-6}, n = 6; K_B = 4.06 ± 0,67 × 10^{-8} M and pA_2 = 7.4 ± 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_2 and 5 % CO_2 to 95 % N_2 and 5 % CO_2 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 µM glibenclamide reduced maximal hypoxic vasorelaxation to 16 ± 3.33 % (Fig. 3a). This confirms earlier findings of other authors and ourselves which show that K_{ATP} channel opening is involved in hypoxic vasodilation. Repaglinide, however, showed a similar effect on hypoxia-induced vasorelaxation in a dose-dependent manner, beginning at 3×10^{-8} M (Fig. 3b). Similar results could be produced by glimepiride. Concentrations of 10^{-7}, 10^{-9} and 3×10^{-9} M glimepiride reduced hypoxic vasorelaxation from a maximum of 41.5 ± 5.12 to 28.3 ± 4.1, 29.5 ± 2.8 and 20.3 ± 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 2×10^{-6} M glibenclamide (Fig. 4a, b). Glimepiride equally showed a dose-dependent inhibition and 3×10^{-6} M glimepiride showed an inhibition of DNP-induced vasorelaxation equal to 2 µM 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. 5×10^{-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_{KATP} 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_{ATP} channel opening during ischaemia has received major attention [2–4, 33, 34]. Several effects of K_{ATP} channel opening were put forward as favourable during

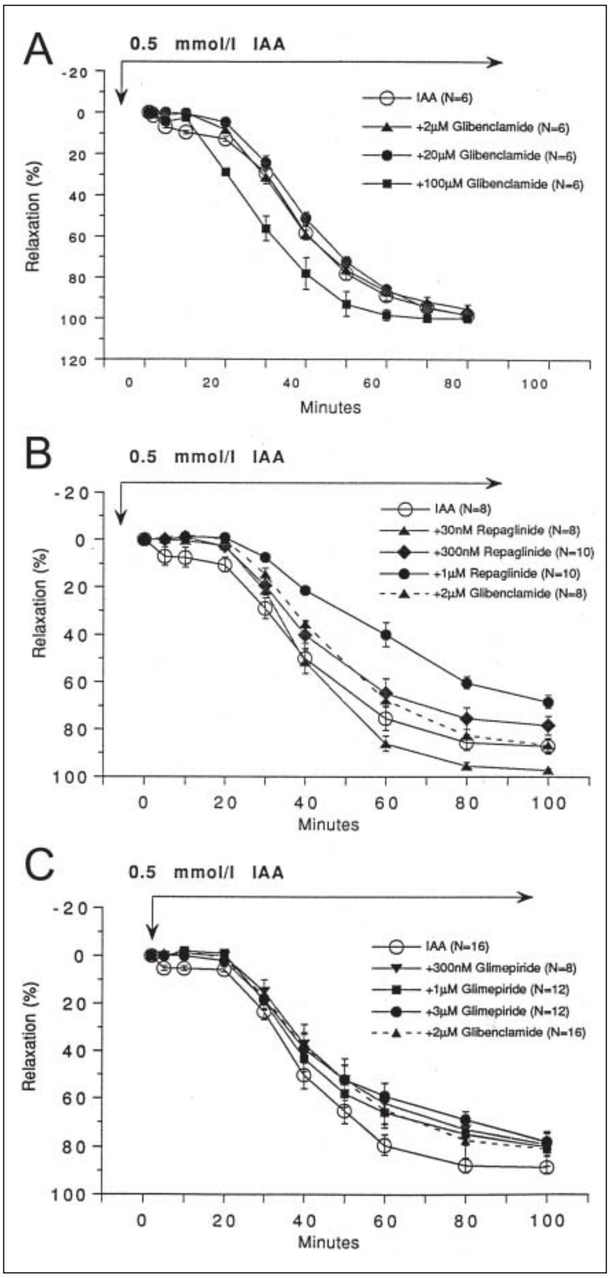

Figure 4. A: Dinitrophenole (1 mM), an uncoupler of oxidative phosphorylation invariably leads to vasodilation, the latter is partially inhibited by glibenclamide in a dose-dependent manner; **B:** Dose dependent inhibition of DNP-induced vasodilation; note repaglinide's intrinsic relaxing activity at higher doses; the inhibition of relaxation is not as pronounced as with glimepiride and glibenclamide; **C:** Inhibition of DNP induced relaxation is maximal under 3 µM glimepiride; the effect of a similar dose of glibenclamide is also introduced in this figure in order to demonstrate the approximate equimolar inhibition; glimepiride is a stronger inhibitor of DNP-induced vasorelaxation than glibenclamide

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.

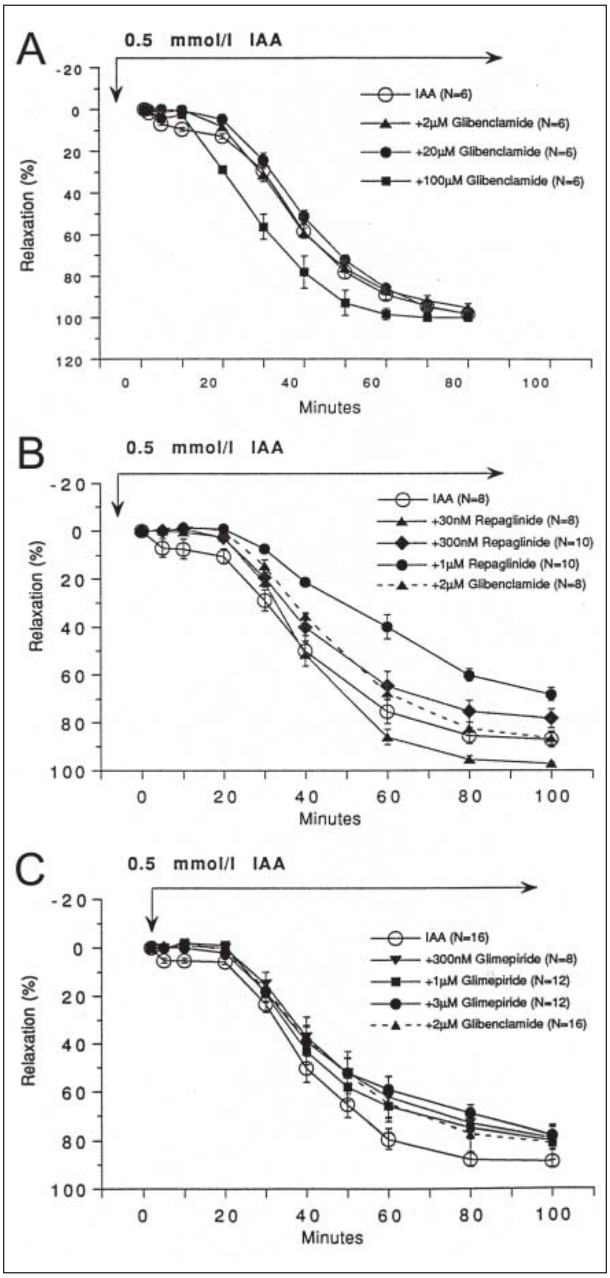

Figure 5. A: Iodoacetic acid (IAA), an inhibitor of the glycolytic enzyme G3P-dehydrogenase leads to vasodilation; inhibition by glibenclamide, repaglinide and glimepiride **(B)** is insignificant; only a dose of 1 μM repaglinide is able to inhibit IAA-induced vasorelaxation after 100 min by 20 %

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 attenuated 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 changes in the configuration of the sulfonylurea receptor. Looking strictly at the coronary response to ischaemia, none of the newly designed sulfonylureas seems to act differently from glibenclamide. From this point of view we cannot support the idea that either should be favoured in patients with coronary heart disease as suggested [21–24]. However, our data do not provide any information on the myocardial actions of these drugs. It would certainly be of interest to look at effects like preconditioning and action potential shortening in detail.

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 provide insight into one particular aspect 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.

References

1. Standen NB. Potassium Channels, Metabolism and Muscle. The G.L. Brown Lecture. Exper Physiol 1992; 77: 1–25.
2. Patel DJ, Purcell HJ, Fox KM on behalf of the CESAR 2 investigation. Cardioprotection by opening of the K_{ATP} channel in unstable angina. Is this a clinical manifestation of myocardial preconditioning? Results of a randomised study with nicorandil. Eur Heart J 1999; 20: 51–7.
3. Di Napoli P, Contegiacomo G, Di Grecchio A, Di Muzio M, Tiloca P, Taccardi AA, Maggi A, Barsotti A. Ischemic preconditioning of rat myocardium: Effects on postischemic coronary endothelium hyperpermeability and microcirculatory damage. J Clin Basic Cardiol 1998; 1: 37–42.
4. Engler RL, Yellon DM. Sulfonylurea K_{ATP} blockade in type II diabetes and preconditioning in cardiovascular disease. Circulation 1996; 94: 2297–301.
5. Gasser R, Köppel H, Horn S, Wallner M, Holzmann S, Klein W. Modulation of glycolysis affects vascular response to sulphonylureas: A study on isolated bovine coronary artery strips. Exp Clin Cardiol 1999; 4: 59–63.
6. Findlay I, Deroubaix E, Guiraudou P, Coraboeuf E. Effects of activation of ATP-sensitive K^+ channels in mammalian ventricular myocytes. Am J Physiol 1989; 257: H1551–H1559.
7. Gasser R, Vaughan-Jones RD. Mechanism of potassium efflux and action potential shortening during ischaemia in isolated mammalian cardiac muscle. J Physiol 1990; 431: 713–41.
8. Wilde AA, Janse MJ. Electrophysiological effects of ATP sensitive potassium channel modulation: implications for arrhythmogenesis. Cardiovasc Res 1994; 28: 16–24.
9. Sobey CG, Woodman OL. Myocardial ischaemia: what happens to the coronary arteries? Trends Pharmacol Sci 1993; 14: 448–53.
10. Beckerath N, Cyrys S, Dischner A, Daut J. Hypoxic vasodilation in isolated, perfused guinea-pig heart: an analysis of the underlying mechanisms. J Physiol 1991; 442: 297–319.
11. Daut J, Maier-Rudolph W, von Beckerath N, Mehrke G, Gunther K, Goedel-Meinen L. Hypoxic vasodilation of coronary arteries is mediated by ATP-sensitive potassium channels. Science 1990; 297: 1341–3.
12. Olsson RA, Bünger R. Metabolic control of coronary blood flow. Progr Cardiovasc Dis 1987; 29: 369–87.
13. Gasser R, Grisold M, Pokan R, Horn E, Horn S, Petnehazy T, Scheidl S, Klein W. Blockade of ATP-dependent K channels in myocardium and coronary artery smooth muscle: a possible cause of increased cardiovascular mortality in sulfonylurea-treated patients. Cardiovasc Drugs Ther 1997; 11: 87–9.
14. University Group Diabetes Program. A study on the effects of agents on vascular complications in patients with adult onset of diabetes II. Mortality results. Diabetes 1970; 19 (Suppl 2): 789–830.
15. Hofmann D, Opie LH. Potassium channel blockade and acute myocardial infarction: implications for management of the non-insulin requiring diabetic patient. Eur Heart J 1993; 14: 1585–9.
16. UKPDS Study Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998; 352: 837–53.
17. Pogatsa G. Possible cardiac benefits of hypoglycaemic sulphonylureas. Cardiovasc Pharmacol 1987; 17: 151–62.
18. Lomuscio A, Vergani D, Marano L, Castagnone M, Fiorentini C. Effects of glibenclamide on ventricular fibrillation in non-insulin dependent diabetics with myocardial infarction. Coron Art Dis 1994; 5: 767–71.
19. Wilde AA. Role of ATP sensitive K^+ channel current in ischemic arrhythmias. Cardiovasc Drug Ther 1993; 7: 521–6.
20. Köppel H, Horn S, Pieber T, Grisold M, Klein W, Gasser R. Observations on the effect of glibenclamide on noninvasive clinical parameters of myocardial ischaemia. Cardiovasc Drug Ther 1998; 12: 383–5.
21. Hobom B. Potassium ion channels – relay stations for insulin secretion, a publication by Hoechst AG, Frankfurt, 1993.
22. Ballagi-Pordany G, Nemeth M, Aranyi Z, Kekesi E, Koltai MZ, Papp G, Pogatsa G. Effect of glimepiride on the electrical activity of isolated rabbit heart muscle. Arzneimittel Forschung/Drug Research 1992; 42: 111–3.
23. Wernicke Panten K, Draeger E, Geisen K. Bietet Glimepirid im Hinblick auf kardiovaskuläre Nebenwirkungen Vorteile gegenüber konventionellen Sulfonylharnstoffen [Is glimepiride better than other sulfonylureas as far as cardiovascular side effects are concerned?]? Diabetes und Stoffwechsel 1995; 4: 32.
24. Smits P, Thien T. Cardiovascular effects of sulphyonylurea derivatives. Implications for the treatment of NIDDM? Diabetologia 1995; 38: 116–21.
25. Wolfenbuttel BHR, Nijst L, Sels J, Menheere P, Müller PG, Nieuwenhuijzen-Krusemann A. Effects of a new oral hypoglycaemic agent, repaglinide, on metabolic control in sulphonylurea-treated patients with NIDDM. Eur J Clin Pharmacol 1993; 45: 113–6.
26. Kukovetz WR, Pöch G, Holzmann S. Cyclic nucleotides and relaxation of vascular smooth muscle. In: Vanhoutte PM, Leusen I (eds). Vasodilation. Raven Press, New York, 1981; 339–53.
27. Kukovetz WR, Holzmann S, Wurm A, Pöch G. Evidence for cyclic GMP-mediated relaxant effects of nitro-compounds in coronary smooth muscle. Naunyn-Schmiedeberg's Arch Pharmacol 1979; 310: 129–38.
28. Gasser R, Köppel H, Brussee H, Grisold M, Holzmann S, Klein W. EDRF does not mediate coronary vasodilation secondary to simulated ischaemia: a study on K_{ATP} channels and N-nitro-L-arginine on coronary perfusion pressure in isolated Langendorff-perfused guinea-pig hearts. Cardiovasc Drug Ther 1998; 12: 279–84.
29. Eisner DA, Nichols CG, O'Neill SC, Smith GL, Valdeolmillos M. The effects of metabolic inhibition of intracellular calcium and pH in isolated rat ventricular cells. J Physiol 1989; 411: 393–418.
30. McDonald T, Mac Leod D. DNP-induced dissipation of ATP in anoxic ventricular muscle. J Physiol 1973; 229: 583–99.
31. Isenberg G, Vereecke J, van der Heyden, Carmeliet E. The shortening of the action potential by DNP in guinea-pig ventricular myocytes is mediated by an increase of a time-dependent K conductance. Pflügers Arch 1983; 397: 251–9.
32. Noma A. ATP regulated K channels in cardiac muscle. Nature 1983; 305: 147–8.
33. Yellon DM, Baxter GF, Garcia-Donado D, Heusch G, Sumeray MS. Ischemic preconditioning: a present position and future directions. Cardiovasc Res 1998; 37: 21–3.
34. Cole WC, Mc Pherson CD, Sontag D. ATP-regulated K-channels protect the myocardium against ischaemia/reperfusion damage. Circ Res 1991; 69: 571–81.
35. Gasser R, Furian C, Grisold M, Dusleag J, Klein W. Myocardial hibernation. Lancet 1992; 340: 796–7.
36. The IONA Study Group. Effect of nicorandil on coronary events in patients with stable angina: The Impact of Nicorandil in Angina (IONA) randomized trial. Lancet 2002; 359: 1269–75.