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VASODILATOR EFFECTS OF PGE_1 IN THE CORONARY AND SYSTEMIC CIRCULATION OF THE RAT ARE MEDIATED BY ATP-SENSITIVE POTASSIUM (K^+) CHANNELS

P. Ney and M. Feelisch

Department of Pharmacology, Schwarz Pharma AG,
Alfred-Nobel-Str. 10, 40789 Monheim, FRG

SUMMARY: This study was undertaken to investigate the possible involvement of K^+ channels in PGE_1-mediated vasodilatation. The increase in coronary flow elicited by PGE_1 in isolated working rat hearts was attenuated by phentolamine and glibenclamide, inhibitors of ATP-regulated K^+ channels, whereas apamin and charybdotoxin, inhibitors of calcium-activated K^+ channels, were ineffective. In the anaesthetized rat, the duration of the hypotensive action of PGE_1 was markedly attenuated by glibenclamide. It is concluded that the vasodilatory action of PGE_1 in the coronary and systemic circulation of the rat is, at least in part, mediated via an opening of ATP-sensitive K^+ channels.

INTRODUCTION

Prostaglandin E_1 (PGE_1) is a potent dilator of coronary arteries and has been demonstrated to increase coronary flow in isolated hearts in vitro. In vivo, application of high doses of this prostaglandin induces systemic hypotension (1). PGE receptors have been identified in membranes of vascular smooth muscle cells (2), and increases in intracellular cAMP levels appeared to be associated with vasodilatation (3). However, the actual signal transduction mechanism is still poorly understood and little is known about the linkage between receptor occupation, cAMP elevation and the biological effect elicited. An opening of potassium (K^+) channels in the membrane of the smooth muscle cell is known to be associated with vascular relaxation (4). Previous investigations revealed that the stable prostacyclin (PGI_2) analogue, iloprost, induces an increase in K^+ permeability and membrane hyperpolarization in the dog carotid artery, suggesting a possible involvement of K^+ channels in prostaglandin-mediated vasodilatation (5). The aim of the present study was, therefore, to elucidate whether or not K^+ channels are involved in the mediation of PGE_1-induced vasodilatation by investigating the possible modulation, by specific inhibitors of different subtypes of K^+ channels, of its functional response in the coronary and systemic circulation of the rat.

Preliminary results of this study have been presented at the 8th International Conference on Prostaglandins and Related Compounds, Montreal, July 26-31, 1992

MATERIALS AND METHODS

Isolated working rat heart: Hearts of male Wistar rats (250-350g) were excised under ether anaesthesia and immediately perfused at constant pressure, according to the working heart technique, with carbogen ($95\%O_2/5\%CO_2$) gassed Krebs-Henseleit solution. The coronary flow (CF) of these spontaneously beating hearts was measured using an electromagnetic flow probe. After equilibration, adenosine (1μM) was infused intracoronarily for 5min to provide a standard vasodilator response. After washout, hearts were challenged with a continuous infusion of PGE_1 (30-50nM) resulting in a 70-80% increase in CF and cumulative concentration-response curves were recorded for phentolamine (0.1-10μM), glibenclamide (1nM-10μM), apamin (0.05-10nM) and charybdotoxin (0.1-10nM). All compounds were continuously infused until equilibrium of the response was achieved (at least 5min). In a second set of experiments, a control concentration-response curve for the coronary dilator effect of PGE_1 (3-100nM) was performed first. Then, the hearts were continuously perfused with glibenclamide (1μM) and a second concentration-response curve for PGE_1 (3nM-1μM) was recorded.

In vivo experiments: Male Wistar rats (300-330g; n=5) were anaesthetized (2mg xylazine/30mg ketamine i.m.) and instrumented with catheters. Blood pressure (BP) and heart rate were measured continuously via a catheter placed in the carotid artery. After recovery of the rats from surgery and stabilization of BP, the hemodynamic response to intraarterial bolus injections of 1-50μg/kg PGE_1 was recorded. Thereafter, 20mg/kg glibenclamide was applied to the rats as an intravenous bolus and a second dose-response curve to PGE_1 was recorded. For comparison of the different treatments, the maximum decreases in BP and the area under the BP curves were calculated.

Calculations and statistics: The data are expressed as mean and standard error (x±SEM) of *n* experiments and animals, respectively. Statistical analysis was performed using the Wilcoxon test. A p value of <0.05 was considered to denote statistical significance.

RESULTS AND DISCUSSION

Perfusion of the isolated working rat heart with PGE_1 (3-75nM) caused a concentration-dependent increase in CF with an EC_{50} of about 15nM (Fig. 2). After infusion of 30nM PGE_1, the increase in CF amounted to 7.0±0.4ml/min, corresponding to a 70-80% increase over basal level (8.6±0.5ml/min, n=15) and this effect was maintained over a period of 60 min (see Fig. 1). A similar effect leading to a maximum dilatation of coronary resistance vessels, indicated by the marked increase in CF, was elicited by infusion of 1μM adenosine. Cumulative addition of phentolamine (0.1-10μM), a nonspecific inhibitor of K^+ channels (6), caused a concentration-dependent attenuation of the PGE_1-elicited response with an IC_{50} of 1μM (n=6). Almost complete inhibition was observed at 10μM phentolamine, suggesting an involvement of K^+ channels in PGE_1-induced coronary vasodilatation. To further characterize the type of K^+

channel involved, more specific inhibitors were applied. The venom toxins apamin and charybdotoxin are known to act as specific inhibitors of low and high conductance calcium-activated K^+ channels, respectively, when applied at low nanomolar concentrations (7). In experiments similar to those depicted in Fig. 1, both toxins did not attenuate the PGE_1-stimulated increase in CF in the concentration range of 0.1-10nM (n=7-8; data not shown). This suggests that the PGE_1-induced increases in CF were not mediated by an opening of calcium-activated K^+ channels. The sulfonylurea compound glibenclamide has been described as a specific inhibitor of ATP-activated K^+ channels (8). Cumulative addition of glibenclamide (1nM-10μM) attenuated the PGE_1-stimulated increase in CF in a concentration-dependent manner (Fig. 1, bottom trace). Half-maximal inhibition was obtained at about 0.1μM glibenclamide (n=9).

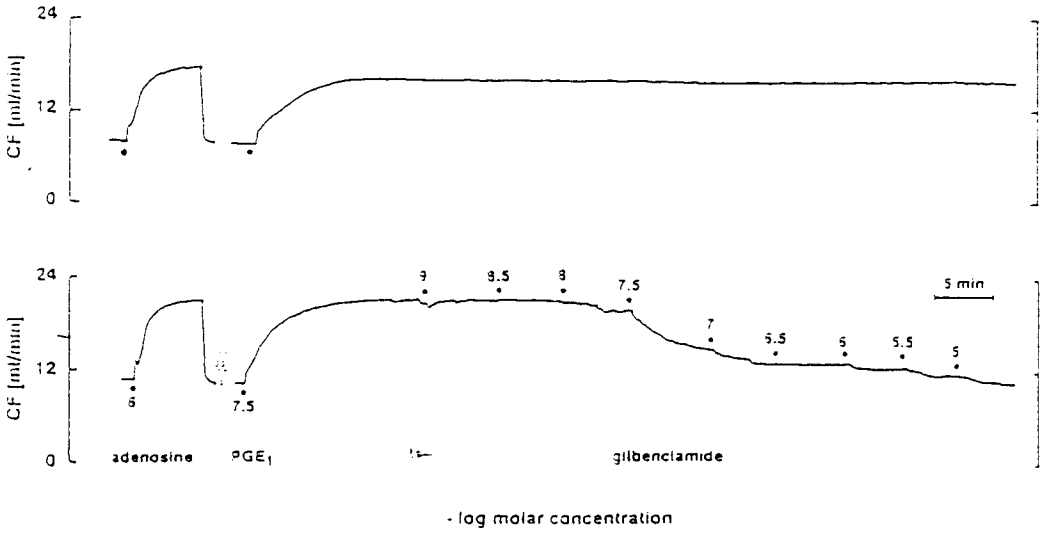

Figure 1. Representative tracing of the effect of PGE_1 (30 nM) on coronary flow at the isolated perfused working rat heart (upper trace) and concentration-dependent inhibition of the PGE_1-mediated increase by glibenclamide (bottom trace).

To elucidate the role of glibenclamide-sensitive K^+ channels in the maintenance of basal CF and to further investigate their contribution to the PGE_1-induced vasodilatation, hearts were continuously perfused with glibenclamide (1μM). This lead to an only 10-20% reduction of basal CF (data not shown). Under the same conditions, the maximum increase in CF elicited by PGE_1 was, however, markedly diminished and the concentration-response curve to PGE_1 was shifted to the right. Even at micromolar concentrations of PGE_1, an only 40-50% increase in CF was achieved (Fig. 2). Taken together, since glibenclamide was more effective than the nonspecific inhibitor phentolamine and the venom toxins tested were ineffective, these results indicate the involvement of ATP-sensitive K^+ channels in PGE_1-elicited coronary vasodilatation. These data confirm and extend recent results obtained with iloprost and PGI_2 at the isolated Langendorff-perfused rabbit heart (9).

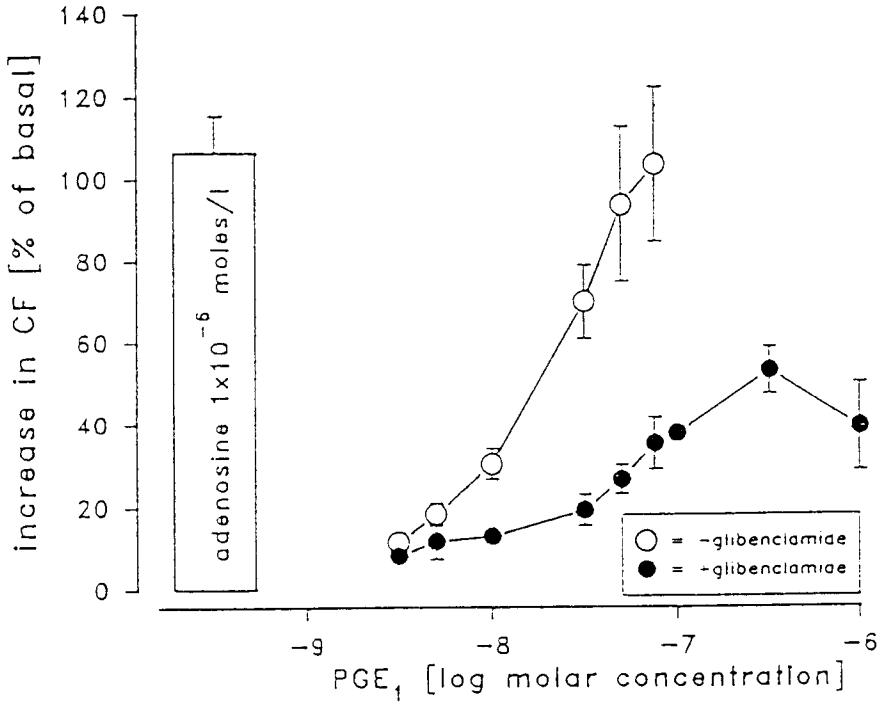

Figure 2. Attenuation of the PGE_1-induced increase in coronary flow at the isolated perfused working rat heart by coinfusion of 1 μM glibenclamide.(n = 3-8)

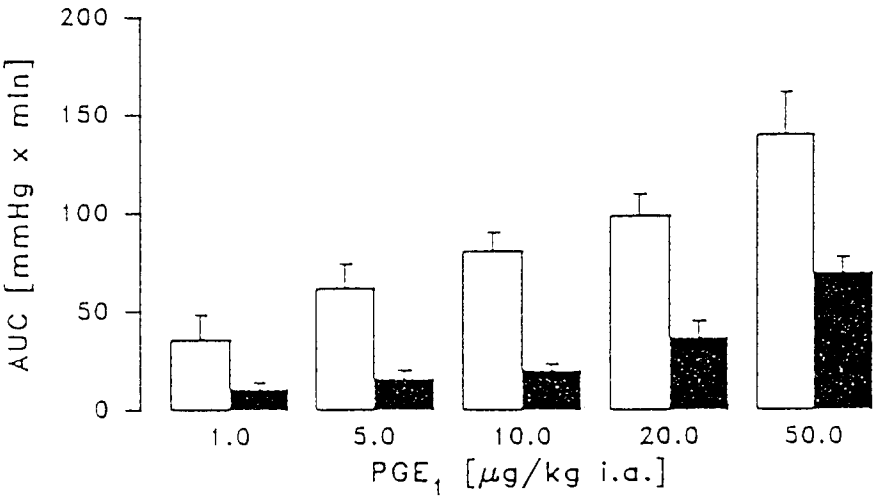

Figure 3. Dose-dependent hypotensive action of PGE_1 (open bars) and its attenuation ($p<0.05$) by pretreatment with an intravenous bolus injection of 20mg/kg glibenclamide (filled bars) (n = 5).

In order to investigate whether or not these channels are also involved in the hypotensive action of PGE_1 in vivo, the effect of a bolus injection of glibenclamide on PGE_1-elicited changes in BP was tested in the anaesthetized rat. Injection of PGE_1 (1-50µg/kg i.a.) caused a transient dose-dependent decrease in BP by 15-35mmHg. The time needed for recovery to baseline increased with increasing doses from 2 to 10min for 1 and 50µg/kg PGE_1, respectively. After intravenous application of glibenclamide (20mg/kg), which itself exerted no consistent effect on BP, the maximum hypotensive responses to PGE_1 were only marginally affected. However, the duration of action of PGE_1 was shortened considerably after pretreatment with glibenclamide. Calculation of the area under the BP curves showed that the application of PGE_1 (1-50µg/kg) induced a dose-dependent increase in the pressure-time product (Fig. 3). After pretreatment of the animals with glibenclamide, the hypotensive response to PGE_1 was markedly attenuated as demonstrated by a significant reduction by 50-70% of the pressure-time product at all doses ($p<0.05$), suggesting that the vasodilatory effect of PGE_1 in vivo is also mediated by glibenclamide-sensitive K^+ channels.

In summary, these data demonstrate a potent and concentration-dependent dilation of the coronary vascular bed by PGE_1 as indicated by the marked increase in CF at the isolated perfused working rat heart. This action was attenuated by phentolamine, a nonspecific inhibitor of K^+ channels. Glibenclamide, a specific inhibitor of ATP-regulated K^+ channels, was even more effective on a molar basis, whereas apamin and charybdotoxin, which are specific inhibitors of low and high conductance calcium-activated K^+ channels, were ineffective as blockers of PGE_1-induced coronary dilatation. In the anaesthetized rat, the dose-dependent hypotensive action of PGE_1 was also considerably attenuated by pretreatment with glibenclamide. Therefore, it is concluded that the vasodilatory action of PGE_1 in the coronary and systemic circulation of the rat is, at least in part, mediated via an opening of ATP-sensitive K^+ channels.

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REFERENCES

1. Nakano J. Cardiovascular actions. In: The Prostaglandins. Ramwell PW, editor. New York: Plenum Press, 1973: 239-316

2. Rücker W, Schrör K. Evidence for high affinity prostacyclin binding sites in vascular tissue: Radioligand studies with a chemically stable analogue. Biochem Pharmacol 1983; 32:2405-10.

3. Ito T, Ogawa K, Enomoto I, Hashimoto H, Kai I, Satake T. Comparison of the effects of PGI_2 and PGE_1 on coronary and systemic hemodynamics and coronary arterial cyclic nucleotide levels in dogs. Adv Prostaglandin Thromb Leukotriene Res 1980; 7:641-6

4. Weston AH. Antihypertensive agents which open smooth muscle K channels. In: Handbook of Experimental Pharmacology, Vol 93. Ganten D, Mulrow PJ, editors. Heidelberg, Springer Verlag, 1990: 643-76

5. Siegel G, Carl A, Adler A, Stock G. Effect of the prostacyclin analogue iloprost on K^+ permeability in the smooth muscle cells of the canine carotid artery. Eicosanoids 1989; 2:213-22

6. McPherson GA, Angus JA. Phentolamine and structurally related compounds selectively antagonize the vascular actions of the K^+ channel opener, cromakalim. Br J Pharmacol 1989; 97:941-9

7. Strong PN. Potassium channel toxins. Pharmac Ther 1990; 46:137-62

8. Sturgess NC, Ashford MLJ, Cook DL, Hales CN. The sulfonylurea receptor may be an ATP-sensitive potassium channel. Lancet 1985; 8453:474-5

9. Jackson WF, König A, Dambacher T, Busse R. Prostacyclin-induced vasodilation in rabbit heart is mediated by ATP-sensitive potassium channels. Am J Physiol 1993; 264:H238-43