Br. J. Pharmac. (1980), **68**, 71–73

DIPYRIDAMOLE: A POTENT STIMULATOR OF PROSTACYCLIN (PGI_2) BIOSYNTHESIS

K.-E. BLASS, H.-U. BLOCK, W. FÖRSTER & K. PÖNICKE

Department of Pharmacology and Toxicology, Martin-Luther-University, Halle (Saale), GDR

1 Dipyridamole (0.01 to 0.75 mM) increased prostacyclin (PGI_2) biosynthesis from tritiated arachidonic acid in rat stomach fundus homogenates by 21 to 350%. The transformation of prostaglandin H_2 (PGH_2) to PGI_2 by a microsomal fraction of pig aorta was stimulated by dipyridamole at 0.1 M by 63%.

2 In the isolated perfused heart of the rabbit dipyridamole at 1 and 5 μg/ml increased PGI_2 release by 70% and 146% respectively.

3 Our results show a stimulation of the second step in PGI_2 biosynthesis (from endoperoxides) by dipyridamole. This effect should be considered in relation to the therapeutic usage of the drug in myocardial infarction.

Introduction

The recently discovered prostacyclin (PGI_2) (Moncada, Gryglewski, Bunting & Vane, 1976) is the major prostaglandin released from the heart (De Deckere, Nugteren & Ten Hoor, 1977). It is also the most potent anti-aggregatory substance known (Moncada *et al.*, 1976) and appears to have a more pronounced lysosomal stabilizing effect than prostaglandin E_1 (PGE_1) (Lefer, Ogletree, Smith, Silver, Nocolaou, Barnette & Gasic, 1978). According to current prostaglandin research, it seems possible that myocardial infarction could be prevented either by inhibition of thromboxane A_2 (TXA_2) formation and/or action, or by increased formation of PGI_2. While the number of drugs known to inhibit TXA_2 is increasing steadily there is little information on the PGI_2 stimulating activities of drugs. During a systematic investigation of cardiovascular drugs (Förster & Pönicke, 1978) dipyridamole was found to be a potent stimulator of PGI_2 biosynthesis.

Methods

PGI_2 biosynthesis was studied using homogenates of rat stomach fundus (1:10, 0.05 M phosphate buffer, pH 7.4, 20 mM disodium edetate (EDTA)). Aliquots were pre-incubated with dipyridamole (0.01 mM, 0.05 mM, 0.2 mM and 0.75 mM) for 5 min at 0°C. Dipyridamole was taken from a stock solution in 60% ethanol; complete solubility of dipyridamole in the homogenate is not guaranteed. After addition of arachidonic acid (5.82 μM, 90 nCi of [5,6,8,9,11,12,14,15-^{3}H]-arachidonic acid per sample) the samples were incubated for 20 min at 37°C. The mixture was acidified to pH 3.0 and the prostaglandins extracted with ethyl acetate. The organic phase was separated, washed to neutrality and evaporated to dryness. The residue was redissolved in ethyl acetate and an aliquot was transferred to a thin layer chromatography (t.l.c.) plate (Silufol-plates from Kavalier, Czechoslovakia). After development in the organic layer of ethyl acetate: acetic acid: trimethylpentane: water, 110:10:20:100, v/v/v/v, the plate was cut into strips (0.5 or 1 cm) and transferred to scintillation vials (relative mobilities were: 6-keto-$PGF_{1\alpha}$ = 0.17, $PGF_{2\alpha}$ = 0.28, PGE_2 = 0.42). Two ml dioxane and 10 ml toluol-based scintillation solution were added and the radioactivity was determined. PGI_2 was determined by measuring its metabolite 6-keto-$PGF_{1\alpha}$.

The enzymatic transformation of PGH_2 to PGI_2 by pig aortic microsomes was studied. Lyophilized pig aortic microsomes (5 or 10 mg), prepared according to Gryglewski, Bunting, Moncada, Flower & Vane (1976), were suspended in 5 ml potassium phosphate buffer (0.025 M, pH 7.5) containing dipyridamole (0.1 mM). The mixture was kept at 0°C for 10 min, then at 37°C for 5 min and subsequently 10 μg PGH_2 in acetone (20 to 40 μl containing $PGF_{1\alpha}$ as internal standard) was added. After an incubation period of 20 min, the mixture was acidified and extracted twice with ethyl acetate. T.l.c. with Kieselgel G (E. Merck, Darmstadt) plates was carried out as described above. The material extracted from the t.l.c. plates (PGF and 6-keto-$PGF_{1\alpha}$ zones) was treated successively with

0007-1188/80/010071-03 $01.00

© Macmillan Journals Ltd 1980

diazomethane, methoxyamine hydrochloride and bis(-trimethylsilyl)-trifluoracetamide; 6-keto-$PGF_{1\alpha}$ was analysed by gas-liquid chromatography (g.l.c.) (1% SE-30 on Gaschrom Q 100-120 mesh, FID, carbon value of $PGF_{1\alpha}$ Me/TMS: 24.4 and of 6-keto-$PGF_{1\alpha}$ Me/MO/TMS: 25.4; the syn and anti forms of 6-keto-$PGF_{1\alpha}$ Me/MO/TMS are not resolved under our g.l.c. conditions).

Rabbits weighing approx. 2 kg were heparinized and killed by a blow on the neck. The isolated heart preparations were perfused with Tyrode solution at 37°C, 8 kPa, gassed with O_2 according to the Langendorff technique. The hearts were allowed to equilibrate for 30 min and control samples for PGI_2 estimation and control coronary flow were taken. Then the perfusion medium was rapidly changed to Tyrode solution containing 1 or 5 μg/ml dipyridamole and 2 min later new samples for PGI_2 estimation and coronary flow were taken. PGI_2 release was estimated by the collection of 15 ml effluents in 1.5 ml 0.1 N NaOH and cooled to 5°C. Dipyridamole was removed by extraction with ether. After rapid ether extraction at pH 5.6 (recovery of PGI_2 50%) the dried samples were redissolved in 200 μl NaCl-carbonate solution (pH 8.9) and the PGI_2-like activity was determined by measurement of the inhibition of adenosine diphosphate (ADP) induced human platelet aggregation.

The authentic prostaglandins (PGI_2 and 6-keto-$PGF_{1\alpha}$) were obtained from Dr E. Pike (Upjohn Company, Kalamazoo, U.S.A.). Dipyridamole was obtained from VEB Arzneimittelwerk Dresden. Student's t test for paired data was used.

Results

In an initial series of experiments, dipyridamole at a concentration of 0.75 mM increased the biosynthesis of PGI_2 by rat stomach homogenates by 350 ± 44% ($n = 12$, $P < 0.001$). In a second series the stimulation of biosynthesis was found to be dose-dependent, 0.01 mM causing an increase of 21 ± 7% ($n = 4$), 0.05 mM an increase of 51 ± 7% ($n = 4$) and 0.20 mM an increase of 95 ± 11% ($n = 4$). The stimulant effect of dipyridamole on PGI_2 biosynthesis was substantially higher than that of any other substance investigated. For example, adrenaline (0.75 mM) stimulated synthesis by 182 ± 31% ($P < 0.01$, $n = 9$).

To differentiate between stimulation of cyclo-oxygenase and PGI_2-synthetase, we investigated the specific transformation of PGH_2 to PGI_2 by aortic microsomes of the pig. Dipyridamole in a concentration of 0.1 mM stimulated this transformation by 63 ± 21% ($P < 0.001$, $n = 10$). There was no significant stimulation by 1 mM adrenaline (+12 ± 12%, $n = 6$).

In addition, we carried out a number of experiments to see whether the enzymatic effect was also demonstrable in the isolated perfused Langendorff heart preparation. Perfusion with 1 or 5 μg/ml dipyridamole caused a slight, but significant, increase in coronary flow (by 17 and 20%, respectively) and augmented the release of PGI_2 by 70 and 146%, respectively (Table 1).

Discussion

The coronary dilator effect of dipyridamole (Kadatz, 1959) seems likely to be correlated with inhibition of adenosine diffusion (Kübler & Bretschneider, 1964). On the other hand, dipyridamole-stimulated PGI_2 formation indicates that this substance may also be involved. A concentration-dependent anti-aggregatory effect of dipyridamole has been observed in man (Rajah, Crow, Penny, Ahmad & Watson, 1977) but was not found in human platelet-rich plasma *in vitro* (Philp, Francey & McElroy, 1973). Our present results show a stimulation of the second step in PGI_2

Table 1 Prostacyclin (PGI_2) release and coronary flow in isolated perfused hearts of rabbits before, and during, perfusion with dipyridamole (1 and 5 μg/ml)

	Before dipyridamole	*During dipyridamole* (1 μg/ml)	*Level of significance*	*Before dipyridamole*	*During dipyridamole* (5 μg/ml)	*Level of significance*
PGI_2 release (ng min^{-1} g^{-1})	0.70 ± 0.15 ($n = 9$)	1.19 ± 0.27 ($n = 9$)	$P < 0.05$	1.01 ± 0.09 ($n = 17$)	2.48 ± 0.29 ($n = 17$)	$P < 0.001$
Coronary flow (ml min^{-1} g^{-1})	3.8 ± 0.4 ($n = 9$)	4.4 ± 0.3 ($n = 9$)	$P < 0.01$	4.5 ± 0.4 ($n = 20$)	5.4 ± 0.2 ($n = 20$)	$P < 0.01$

Mean Values ± s.e. mean are shown. Significance of difference was measured by Student's t test for paired data.

synthesis (from endoperoxides) by dipyridamole, indicating that this possible mechanism of action of the drug should be considered.

The authentic prostaglandins (PGI_2 and 6-keto-$PGF_{1\alpha}$) were a generous gift from Dr J. E. Pike, Upjohn Co., Kalamazoo, U.S.A.

References

De Deckere, E.A.M., Nugteren, D.H. & Ten Hoor, F. (1977). Prostacyclin is the major prostaglandin released from the isolated perfused rabbit and rat heart. *Nature, Lond.*, **268**, 160–163.

Förster, W. & Pönicke, K. (1978). The influence of cardiovascular drugs on the biosynthesis of prostacyclin. Poster, *7th International Congress of Pharmacology*, Paris, 1978.

Gryglewski, R.J., Bunting, S., Moncada, S., Flower, R.J. & Vane, J.R. (1976). Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin—X) which they make from prostaglandin endoperoxides. *Prostaglandins*, **12**, 685–713.

Kadatz, R. (1959). Die pharmakologischen Eigenschaften der neuen coronarerweiternden Substanz 2,6-Bis(diaethenolamino-4,8-dipiperidono-pyrimido(5,4-d)pyrimidin. *Arzneimittel Forsch.*, **9**, 39–45.

Kübler, W. & Bretschneider, H.J. (1964). Kompetitive Hemmungen der katalysierten Adenosindiffusion als Mechanismus der coronarerweiternden Wirkung eines Pyrimido-pyrimidin-Derivates. *Pflügers Arch.*, **280**, 141–157.

Lefer, A.M., Ogletree, M.L., Smith, J.B., Silver, M.J., Nicolaou, K.C., Barnette, W.E. & Gasic, G.P. (1978). Prostacyclin: A potentially valuable agent for preserving myocardial tissue in acute myocardial ischemia. *Science, New York*, **200**, 52–54.

Moncada, S., Gryglewski, R., Bunting, S. & Vane, J.R. (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature, Lond.*, **263**, 663–665.

Philp, R.B., Francey, I. & McElroy, F. (1973). Effects of dipyridamole and five related agents on human platelet aggregation and adenosine uptake. *Thrombosis Res.*, **3**, 35–50.

Rajah, S.M., Crow, M.J., Penny, A.F., Ahmad, R. & Watson, D.A. (1977). The effect of dipyridamole on platelet function: correlation with blood levels in man. *Br. J. clin. Pharmac.*, **4**, 129–133.

(*Received January 25, 1978. Revised March 27, 1978.*)