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(5Z)-carbacyclin discriminates between prostacyclin-receptors coupled to adenylate cyclase in vascular smooth muscle and platelets

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1 (5E)- and (5Z)-carbacyclin are prostacyclin (PGI_2) analogues endowed with antiaggregating and vasodilator properties, which stimulate adenylate cyclase activity in membranes from human platelets and cultured myocytes from rabbit mesenteric artery.

2 In platelets they display the same efficacy as prostaglandin E_1 (PGE_1), and hence PGI_2, both as activators of adenylate cyclase and as inhibitors of aggregation.

3 In contrast, in vascular smooth muscle cells (5Z)-carbacyclin fails to produce the same degree of stimulation of the enzyme as PGI_2, (5E)-carbacyclin and PGE_1, nor does it induce the maximal relaxation of the mesenteric artery as do the other prostaglandins.

4 (5Z)-carbacyclin is also able to antagonize the activation of adenylate cyclase and the relaxation elicited by PGE_1 or PGI_2 in the mesenteric artery, and therefore it displays partial agonist properties in these cells.

5 We conclude that the receptors for PGI_2 coupled to adenylate cyclase in platelets and vascular smooth muscle cells are different from each other, because (5Z)-carbacyclin can discriminate between them, being a partial agonist at myocyte but not at platelet level.

Introduction

Prostacyclin (PGI_2) is the most potent inhibitor of platelet aggregation (Moncada *et al.*, 1976) and, because of this property, it has many potential clinical applications. However, prostacyclin has some drawbacks which have so far limited a more widespread clinical use. In fact, not only is it chemically and metabolically unstable (Moncada *et al.*, 1976; Johnson *et al.*, 1976), but it also displays potent vasodepressor actions (Moncada *et al.*, 1976; 1978), when often only the anti-platelet effect is desired.

For these reasons, a great effort has been made toward the development of PGI_2 analogues more suitable for clinical use (Whittle & Moncada, 1984). The design of a PGI_2 analogue endowed with a high degree of selectivity for the platelet receptor will be successful only if this receptor differs from that on vasculature.

Because both inhibition of platelet aggregation and vasodilatation are supposed to be mediated by an increase in intracellular adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels (Gorman *et al.*, 1977; Tateson *et al.*, 1977; Miller *et al.*, 1979; Kukovetz *et al.*, 1979; Lombroso *et al.*, 1984; Oliva *et al.*, 1984a,b), through activation of adenylate cyclase (AC), we have addressed the problem of characterization of the platelet and vascular receptors for PGI_2 by investigating the activation of this enzyme in membranes from human platelets and from rabbit vascular myocytes. The evaluation in these systems of some prostacyclin analogues (PGE_1, 6ß-PGI_1, 6-keto-PGE_1), so far has not revealed any major difference between the platelet and the vascular receptors for PGI_2 coupled to adenylate cyclase (Lombroso *et al.*, 1984; Oliva *et al.*, 1984 a,b).

We describe here further studies performed with two epimers of carbacyclin (Morton *et al.*, 1979) (Figure 1) which share with PGI_2 the antiaggregating and vasodilator effects (Whittle *et al.*, 1980), and demonstrate that the 5Z epimer is able to discriminate between the platelet and vascular receptor.

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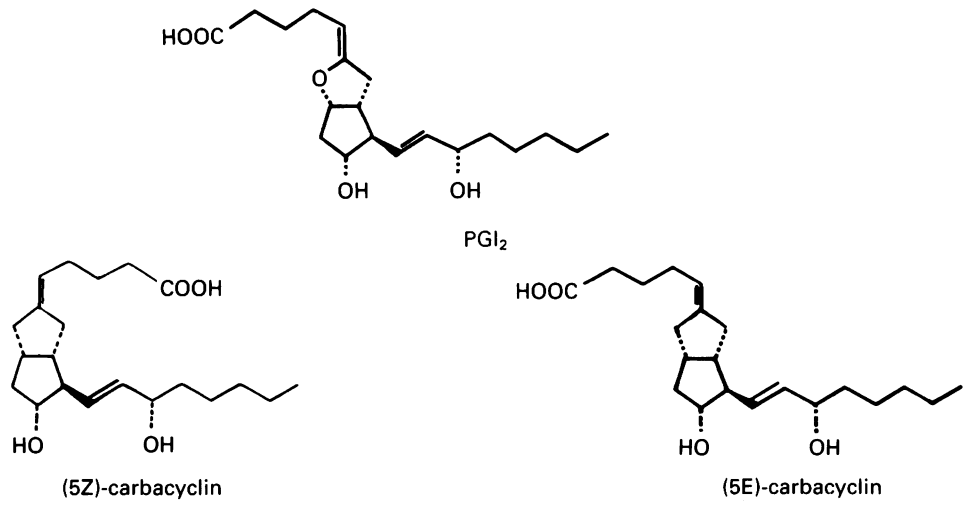

Figure 1 Structures of prostacyclin (PGI_2) and its analogues, the carbacyclins.

Methods

Materials

[8-^{14}C]-adenosine triphosphate ([8-^{14}C]-ATP) and [8-^{3}H]-cyclic AMP were from New England Nuclear, Boston, MA, U.S.A; ATP, cyclic AMP, guanosine triphosphate (GTP), creatine phosphate, creatine phosphokinase and sodium arachidonate were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Prostacyclin (PGI_2), PGE_1, (5E)-carbacyclin and (5Z)-carbacyclin were synthesized by the Upjohn Co., Kalamazoo, MI, U.S.A., and supplied by The Wellcome Research Laboratories, Beckenham, U.K. The solutions of PGI_2, which was stored in ethanol at $-20°C$, were freshly prepared immediately before use in 10 mM Tris-HCl buffer, pH 8. The other prostaglandins were dissolved in the same Tris buffer. In the experiments with myocyte membranes, where the high concentrations of (5Z)-carbacyclin gave solubility problems, it was more convenient to dissolve and dilute this prostaglandin, and therefore also the others, with 40% ethanol in Tris buffer (to yield a final ethanol concentration of 4% in the sample). The inclusion of ethanol in the adenylate cyclase assay did not modify the pattern of response to the prostaglandins. Eagle's minimum essential medium F11, foetal calf serum, trypsin-EDTA, penicillin (10,000 u ml^{-1}), streptomycin (10 mg ml^{-1}), tricine buffer (1 M) and non-essential amino acids (100 ×) were purchased from Grand Island Biological Co., Madison, WI, U.S.A.; disposable culture flasks and petri dishes were from Corning Glassworks, Amedfield, MA, U.S.A.

Cell cultures

Male white New Zealand rabbits (2–3 kg) were used. Cultures of smooth muscle cells from intima-medial layer of rabbit aorta and mesenteric arteries were prepared according to the method of Ross (1971), as previously described by Oliva *et al.* (1984b).

Preparation of membranes

Platelet concentrates (collected in citric acid/sodium citrate/sodium phosphate/dextrose) from 3–4 healthy male volunteers were pooled. A crude membrane preparation (pellet at 27,000 *g*) was prepared as described by Lombroso *et al.* (1984).

Smooth muscle cell monolayers from rabbit mesenteric artery (used between the 8th and 14th passage) were washed in 50 mM Tris-HCl buffer (pH 7.4), harvested by scraping, pooled and the membrane preparation (pellet at 15,000 *g*) was obtained as described by Oliva *et al.* (1984b).

Platelet aggregation studies

Blood was collected in 3.8% sodium citrate (9:1). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared as previously described (Tremoli *et al.*, 1979). Platelet count was adjusted to 300–400,000 μl^{-1} by adding PPP. Prostaglandin inhibition of platelet aggregation was measured in PRP samples stimulated by collagen 5 ng μl^{-1} using an ELVI Logos aggregometer by the turbidimetric technique of Born (1962).

Rabbit mesenteric artery studies

Spiral strips of rabbit mesenteric artery were prepared and set up using the laminar-flow technique of Ferreira & Costa (1976). The spirals were superfused with Krebs solution of the following composition ($g l^{-1}$): NaCl 6.9, KCl 0.35, KH_2PO_4 0.16, $MgSO_4.7H_2O$ 0.29, $CaCl_2$ 0.28, glucose 1 and $NaHCO_3$ 2.1. Flow rate was 0.2 ml min^{-1}, the buffer was kept at 37°C and gassed with 95% O_2 and 5% CO_2. The resting tone of the preparations was 1 g and contractions were recorded with isotonic transducers Mod. 7006 (Ugo Basile, Comerio, Italy) connected to a 2 channel Gemini recorder, Mod. 7070 (Ugo Basile). The tissues were equilibrated for 2 h and subsequently challenged with bolus injections of different agonists; the volume of the injected bolus was 0.1 ml.

Adenylate cyclase assay

The standard assay mixture (final volume: 100 µl) contained: 10 mM Tris-HCl buffer (pH 8); 0.10 mM [8-^{14}C]-ATP (50 dpm $pmol^{-1}$); 0.5 mM [8-^{3}H]-cyclic AMP (approximately 360 dpm $nmol^{-1}$); 2 mM $MgCl_2$; 2 mM creatine phosphate; 17 u ml^{-1} creatine phosphokinase; 10 µM GTP and the indicated prostaglandins. The incubation, started with the addition of the membrane preparation (0.04–0.09 and 0.06–0.10 mg protein per sample for platelet and mesenteric membranes respectively), was carried out at 30°C for 8 min. [8-^{3}H]-cyclic AMP was included in the assay mixture to permit correction for column loss and for the possible effect of phosphodiesterases (Katz *et al.*, 1978) which in any case was almost negligible.

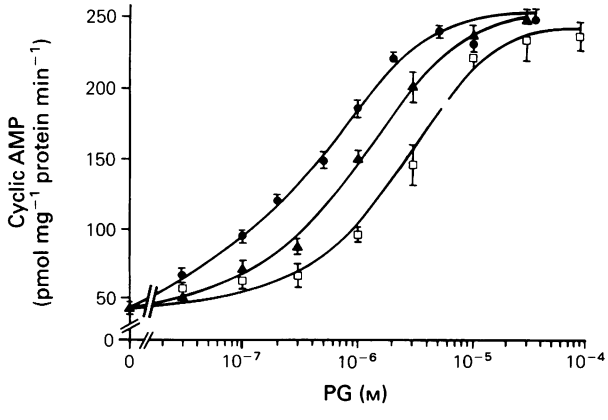

Figure 2 Dose-response curves for the activation of adenylate cyclase by different prostaglandins in membranes of human platelets: (●) (5E)-carbacyclin; (▲) prostaglandin E_1; (□) (5Z)-carbacyclin.

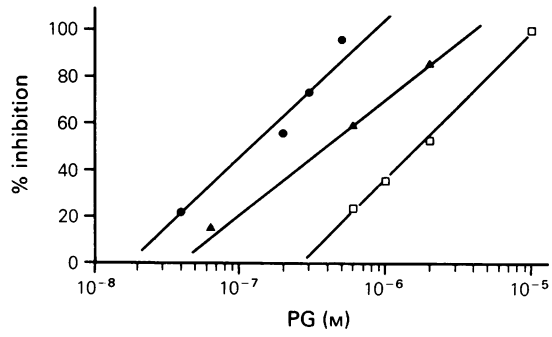

Figure 3 Dose-response curves for the inhibition of human platelet aggregation induced by collagen (5 ng μl^{-1}): (●) (5E)-carbacyclin; (▲) prostaglandin E_1; (□) (5Z)-carbacyclin.

[8-^{14}C, 8-^{3}H]-cyclic AMP was isolated and detected according to Salomon *et al.* (1974). Protein concentrations were determined according to Bradford (1976).

Expression of results

The results are expressed as mean ± s.d. of triplicate determinations in one experiment, performed at least three times with equivalent results.

Results

Human platelets

The effect of increasing concentrations of (5E)-carbacyclin and (5Z)-carbacyclin on AC activity was investigated in human platelet membranes and compared with that of PGE_1. Figure 2 shows that all the prostaglandins were able to stimulate AC in a dose-dependent fashion with approximately parallel curves, and that they were equieffective (maximal stimulation: 5 fold). The concentrations eliciting half-maximal stimulation (EC_{50}) were 0.307 ± 0.162 µM, 0.633 ± 0.351 µM and 2.83 ± 0.29 µM for (5E)-carbacyclin, PGE_1 and (5Z)-carbacyclin, respectively.

(5Z)-carbacyclin was tested for its ability to inhibit collagen-induced platelet aggregation (Figure 3) and was found to induce the same maximal response as PGE_1 and (5E)-carbacyclin (100% inhibition). The concentrations eliciting half-maximal inhibition (IC_{50}) were 105 ± 32 nM, 145 ± 153 nM, and 1.96 ± 0.99 µM, for (5E)-carbacyclin, PGE_1 and (5Z)-carbacyclin, respectively, in agreement with the results of Whittle *et al.* (1980).

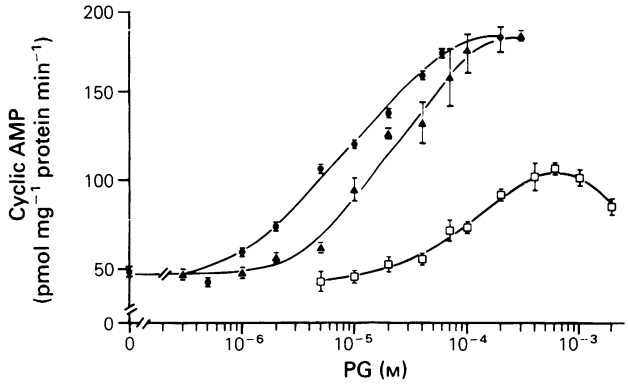

Figure 4 Dose-response curves for the activation of adenylate cyclase by different prostaglandins in membranes of myoctyes from rabbit mesenteric artery: (●) (5E)-carbacyclin; (▲) prostaglandin E_1; (□) (5Z)-carbacyclin.

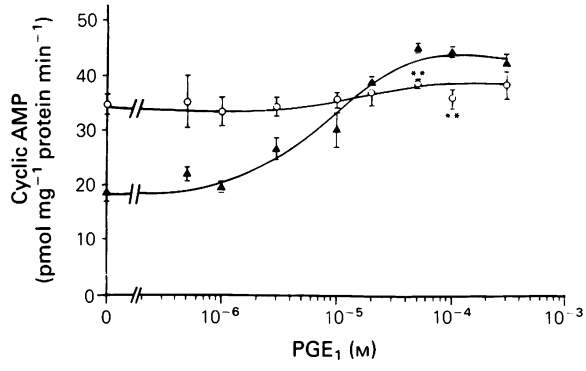

Figure 6 Dose-dependent effect of prostaglandin E_1 (PGE_1) on adenylate cyclase activity of myoctyes in the absence (▲) and the presence (O) of (5Z)-carbacyclin, 0.2 mM. $^{**}P < 0.001$ when compared with corresponding concentrations of PGE_1 alone.

Rabbit mesenteric arterial myocytes

In membranes of cultured smooth muscle cells from rabbit mesenteric artery (5E)-carbacyclin, PGE_1 and (5Z)-carbacyclin stimulated AC in a dose-dependent fashion (Figure 4). However, at variance with the results obtained in human platelet membranes, the maximal stimulation attained with the two carbacyclins was different. In fact, (5E)-carbacyclin activated AC to the same extent (3.5 fold) and approximately with the same potency ($EC_{50} = 5.93 \pm 2.10\,\mu M$) as PGE_1 ($EC_{50} = 14.9 \pm 8.6\,\mu M$).

In contrast, (5Z)-carbacyclin failed to reach the same response elicited by the other two prostaglandins, in that the maximal stimulation (attained at 0.4 mM) was 2 fold. (5Z)-carbacyclin had an EC_{50} of 0.104 ± 0.021 mM.

The lower efficacy of (5Z)-carbacyclin suggested that it might act as a partial agonist. In order to evaluate this hypothesis, the effect of increasing concentrations of (5Z)-carbacyclin on PGE_1-stimulated AC activity was investigated. As shown in Figure 5, (5Z)-carbacyclin displayed antagonistic properties in this system, since it was able to reduce the

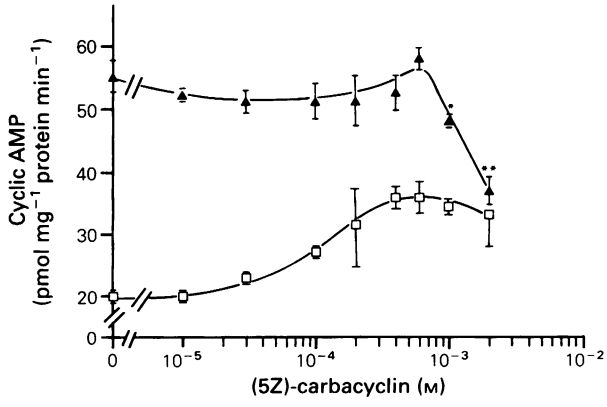

Figure 5 Dose-dependent effect of (5Z)-carbacyclin on adenylate cyclase activity of myocytes in the absence (□) and presence (▲) of prostaglandin E_1 0.3 mM. $^{*}P < 0.01$, $^{**}P < 0.001$ when compared with stimulation by prostaglandin E_1 alone.

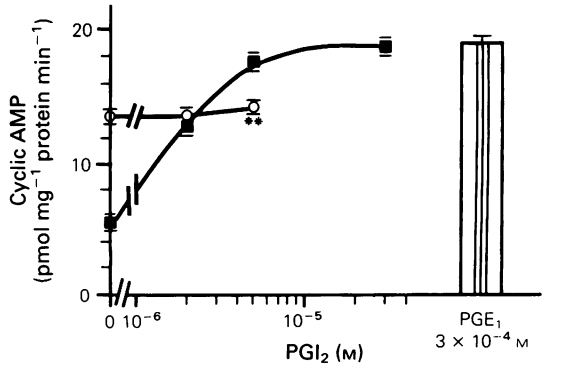

Figure 7 Dose-dependent effect of prostacyclin (PGI_2) on adenylate cyclase activity of myocytes in the absence (■) and presence (O) of (5Z)-carbacyclin 1 mM. $^{**}P < 0.001$ when compared with corresponding concentration of PGI_2 alone. The column represents the stimulation by a maximally effective concentration of prostaglandin E_1 (PGE_1).

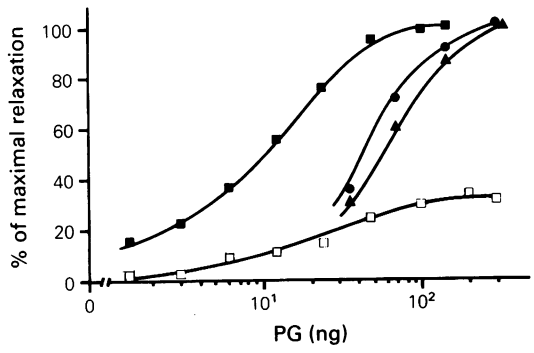

Figure 8 Dose-response curves for the relaxation of rabbit mesenteric artery: 100% relaxation was taken as the maximal effect attainable with prostacyclin (PGI_2); (●) (5E)-carbacyclin; (□) (5Z)-carbacyclin; (▲) prostaglandin E_1; (■) PGI_2.

stimulation of AC induced by 0.3 mM PGE_1. (5Z)-carbacyclin also antagonized the activation of AC elicited by 40 μM (5E)-carbacyclin (data not shown).

The agonist/antagonist properties of (5Z)-carbacyclin are further demonstrated by the experiment of Figure 6, where a dose-response curve for PGE_1 in the presence and absence of a fixed concentration of (5Z)-carbacyclin is shown. As expected from a partial agonist (Ariens *et al.*, 1964), this carbacyclin stimulates AC at low concentrations of the full agonist PGE_1, while it antagonizes the effects of higher concentrations of the latter.

The particular cell line used in the experiment shown in Figure 6 displayed a lower degree of stimulation than those used in other experiments (see, e.g. Figure 4), and therefore the difference between PGI_2 and (5Z)-carbacyclin efficacies appears smaller than in other experiments. However, as already discussed (Oliva *et al.*, 1984b), the pattern of AC stimulation was comparable to that of the other membrane preparations.

The experiment depicted in Figure 7 demonstrates that the partial agonist properties of (5Z)-carbacyclin are displayed not only versus PGE_1, but also versus PGI_2, as one would expect if the two latter prostaglandins act through the same receptor. In fact, a maximally activating concentration of (5Z)-carbacyclin (1 mM) elicited a lower enzyme stimulation than PGI_2 (which was equieffective with PGE_1). Furthermore, this same concentration of (5Z)-carbacyclin was not additive with an equieffective concentration of PGI_2 (2 μM), while it was able to inhibit the enzyme activation elicited by higher concentrations of PGI_2 (5 μM). These results are in line with the theory of partial agonist effects (Ariens *et al.*, 1964).

The effect of increasing amounts of the various prostaglandins in relaxing rabbit mesenteric artery was also investigated. Figure 8 shows that (5Z)-carbacyclin failed to reach the maximal relaxation of rabbit mesenteric artery attained with PGI_2, (5E)-carbacyclin and PGE_1. In fact, at 280 ng, (5Z)-carbacyclin elicited less than 40% of the maximal response.

Again, this result suggested that (5Z)-carbacyclin might be a partial agonist. This is confirmed by the data in Figure 9, which show that this carbacyclin antagonized the effects of PGI_2, decreasing the relaxation elicited by the latter, when they were coadministered. That the lower response was not due to a decreased sensitivity of the mesenteric artery is demonstrated by the unaltered response to the subsequent administration of PGI_2.

Discussion

(5E)- and (5Z)-carbacyclin are two chemically stable analogues of prostacyclin, the former being isosteric with the natural prostaglandin. Both carbacyclins

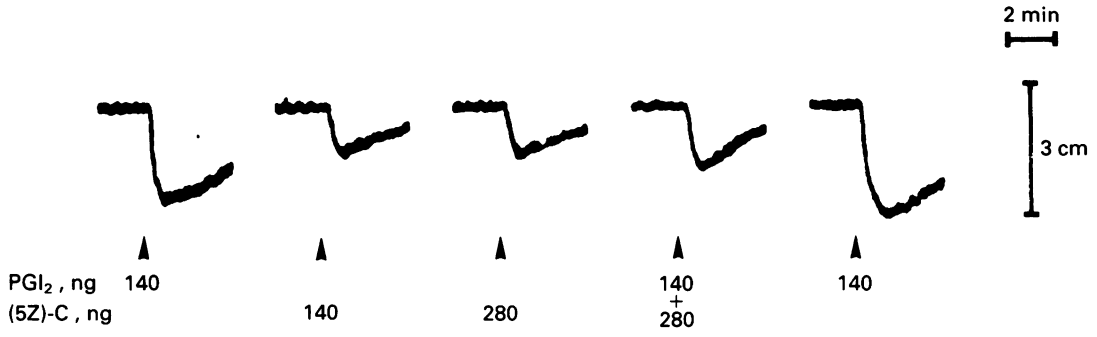

Figure 9 Relaxation of rabbit mesenteric artery by prostacyclin (PGI_2) and (5Z)-carbacyclin ((5Z)-C) alone, and their combined effect.

mimic the effect of PGI_2 and PGE_1, in that they are inhibitors of platelet aggregation *in vitro* and *ex vivo* and they lower arterial blood pressure (Whittle *et al.*, 1980). Since inhibition of platelet aggregation and vasodilatation are supposed to be mediated by increases in intracellular cyclic AMP levels, the study of adenylate cyclase stimulation by these prostaglandins might shed some light on the nature of the receptor involved in these phenomena. We have used PGE_1 as a reference compound in these studies, since we have demonstrated that it displays the same efficacy as prostacyclin, and, by means of binding (Lombroso *et al.*, 1984) or additivity studies (Nicosia *et al.*, 1987), that it acts through identical receptors.

In human platelet membranes, (5E)- and (5Z)-carbacyclins activate adenylate cyclase to the same extent as PGE_1, and therefore as PGI_2. The effect of (5E)-carbacyclin is in agreement with the data of Stein & Martin (1984), and was expected since this prostaglandin raises cyclic AMP levels (Ceserani *et al.*, 1980), and its anti-aggregating effect is potentiated by the phosphodiesterase inhibitor, theophylline (Whittle *et al.*, 1980; Morita *et al.*, 1980).

The stimulation of adenylate cyclase activity correlates well with the functional response of platelets, in that the rank order of potency for enzyme activation is paralleled by that found for inhibition of platelet aggregation (Whittle *et al.*, 1980). Furthermore, PGE_1 and the carbacyclins display parallel dose-response curves and attain the same maximal effect both in adenylate cyclase activation and in platelet aggregation studies.

The pattern is completely different in myocytes cultured from rabbit mesenteric artery. In this system, while (5E)-carbacyclin activates adenylate cyclase to the same extent as PGE_1, the (5Z) epimer has a markedly lower efficacy, the maximal stimulation of the enzyme being only 40–45% of that obtained with the other prostaglandins. It is noteworthy that both in smooth muscle and platelets the enzyme stimulation is in very good aggreement with the biological response; in fact, we demonstrate that (5Z)-carbacyclin is less efficient (40% of maximal) than the other prostaglandins in the relaxation of rabbit mesenteric artery *in vitro*.

The fact that (5Z)-carbacyclin is unable to produce the maximal effect elicited by PGI_2 or PGE_1 could be explained on the basis of two different hypotheses: either (5Z)-carbacyclin interacts with an independent receptor, different from the one shared by prostacyclin and other analogues, and possesses a lower intrinsic activity, or this carbacyclin behaves as a partial agonist at the prostacyclin receptor. That the latter is the more likely hypothesis is demonstrated by the experiments illustrated in Figures 5 to 7, which show that (5Z)-carbacyclin is able to decrease the stimulation of adenylate cyclase elicited by either PGE_1 or PGI_2. This carbacyclin seems therefore to be endowed with antagonistic properties at the PGE_1/PGI_2 receptor, as one would expect from a partial agonist (Ariens *et al.*, 1964).

While it is true that such antagonist properties appear only at relatively high concentrations, the relevance of our finding is supported by similar results which have been obtained recently with another PGI_2 analogue having the same configuration as (5Z)-carbacyclin at carbon 5: in fact, FCE 22176, i.e. (5Z)-13, 14-didehydro-20-methyl-carboprostacyclin, has been shown to be a competitive antagonist of PGI_2 on guinea-pig trachea and atrium (Fassina *et al.*, 1985). These results, taken together with our present findings, suggest that position 5 is a key one in determining the mode of interaction of PGI_2 with its receptors.

Whittle *et al.* (1980, 1984) had investigated the selectivity of a number of PGI_2 analogues, including (5Z)-carbacyclin, by means of the 'selectivity ratio', that is by calculating the ratio of the relative potency of the analogue to prostacyclin as a vasodepressor *in vivo* and a platelet inhibitor *in vitro*. The differences in this index obtained with a series of compounds seemed to indicate that some intrinsic differences exist between the platelet and vascular receptor for PGI_2. However, as the authors themselves pointed out (Whittle *et al.*, 1984), the 'selectivity ratio' must be interpreted with caution, mainly because it is inferred from the comparison of *in vivo* and *in vitro* data, which might be affected by differences in metabolism or pharmacodynamics among the various analogues.

Therefore, our results give evidence, obtained directly at the receptor-binding site level, of the possibility of discriminating between the platelet and vascular PGI_2 receptors, and suggest that the moiety comprising carbon 5 might play an important role in the design of an analogue with a high degree of selectivity between the cardiovascular and anti-platelet effects.

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References

ARIENS, E.J., SIMONIS, A.M. & VAN ROSSUM, J.M. (1964). Drug-receptor interaction: interaction of one or more drugs with one receptor system. In *Molecular Pharmacology*, Vol. 1. ed. Ariens, E.J. pp. 171–180, New York: Academic Press.

BORN, G.V.R. (1962). Aggregation of blood platelets by

adenosine diphosphate and its reversal. *Nature*, **194,** 927–929.

BRADFORD, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72,** 248–254.

CESERANI, R., GROSSONI, M., LONGIAVE, D., MIZZOTTI, B., POZZI, O., DEMBINSKA-KIEC, A. & BIANCO S. (1980). dL-9a-deoxy-9a-methylene-PGI_2 (a stable prostacyclin derivative): preliminary pharmacological data. *Prostaglandins Med.*, **5,** 131–139.

FASSINA, G., FROLDI, G. & CAPARROTTA, L. (1985). A stable isosterically modified prostacyclin analogue, FCE-22176, acting as a competitive antagonist to prostacyclin in guinea-pig trachea and atria. *Eur. J. Pharmac.*, **113,** 459–460.

FERREIRA, S.H. & COSTA, F.S. (1976). A laminar flow technique with much increased sensitivity for the detection of smooth muscle stimulating substances. *Eur. J. Pharmac.*, **39,** 379–381.

GORMAN, R.R., BUNTING, S. & MILLER, O.V. (1977). Modulation of human platelet adenylate cyclase by prostacyclin (PGX). *Prostaglandins*, **13,** 377–387.

JOHNSON, R.A., MORTON, D.R., KINNER, J.H., GORMAN. R.R., McGUIRE, J.C., SUN, F.F., WHITTAKER, N., BUNTING, S., SALMON, J., MONCADA, S. & VANE, J.R. (1976). The chemical structure of prostaglandin x (prostacyclin). *Prostaglandins*, **12,** 915–928.

KATZ, M.S., KELLY, T.M., PINEYRO, M.A. & GREGERMAN, R.I. (1978). Activation of epinephrine and glucagon-sensitive adenylate cyclases of rat liver by cytosol protein factors. Role in loss of enzyme activities during preparation of particulate fractions, quantitation and partial characterization. *J. Cyclic Nucleotide Res.*, **5,** 389–407.

KUKOVETZ, W.R., HOLZMANN, S., WURM, A. & POCH, G. (1979). Prostacyclin increases cAMP in coronary arteries. *J. Cyclic Nucleotide Res.*, **5,** 469–476.

LOMBROSO, M., NICOSIA, S., PAOLETTI, R., WHITTLE, B.J.R., MONCADA, S. & VANE, J.R. (1984). The use of stable prostaglandins to investigate prostacyclin (PGI_2)-binding sites and PGI_2-sensitive adenylate cyclase in human platelet membranes. *Prostaglandins*, **27,** 321–333.

MILLER, O.V., AIKEN, J.W., HEMKER, D.P., SHEBUSKI, R.J. & GORMAN, R.R. (1979). Prostacyclin stimulation of dog arterial cyclic AMP levels. *Prostaglandins*, **18,** 915–925.

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*, **263,** 663–665.

MONCADA, S., FERREIRA, S.H. & VANE, J.R. (1978). Bioassay of prostaglandins and biologically active substances derived from arachidonic acid. In *Advances in Prostaglandin and Thromboxane Research*. volume 5. ed. Frölich J.C. pp. 211–236. New York: Raven Press.

MORITA, A., MORI, M., HASEGAWA, K., KOJIMA, K. & KOBAYASHI, S. (1980). Anti-platelet aggregating and disaggregating activities of 6, 9-methano PGI_2. *Life Sci.*, **27,** 695–701.

MORTON, D.R., BUNDY, G.L. & NISHIZAWA, E.E. (1979). Five-membered ring-modified prostacyclin analogs. In *Prostacyclin*. ed. Vane J.R. & Bergstrom S. pp. 31–41. New York: Raven Press.

NICOSIA, S., OLIVA, D., NOE', M.A., CORSINI, A., FOLCO. G.C. & FUMAGALLI, R. (1987). PGI_2 receptors in vasculature and platelets: 5Z-carbacyclin discriminates between them. In *Advances in Prostaglandin, Thromboxane and Leukotriene Research*, Vol. 16, ed. Samuelsson P., Paoletti, R. & Ramwell P. New York: Raven Press, (in press).

OLIVA, D., BERNINI, F., CORSINI, A. & NICOSIA, S. (1984a). 6-keto-prostaglandin E_1-sensitive adenylate cyclase and binding sites in membranes from platelet and cultured smooth muscle cells. *Biochem. Pharmac.*, **33,** 3755–3758.

OLIVA, D., NOE', A., NICOSIA, S., BERNINI, F., FUMAGALLI, R., WHITTLE, B.J.R., MONCADA, S. & VANE, J.R. (1984b). Prostacyclin-sensitive adenylate cyclase in cultured myocytes: differences between rabbit aorta and mesenteric artery. *Eur. J. Pharmac.*, **105,** 207–213.

ROSS, R. (1971). The smooth muscle cell. *J. cell. Biol.*, **50,** 172–186.

SALOMON, Y., LONDOS, C. & RODBELL, M. (1974). A highly sensitive adenylate cyclase assay. *Anal. Biochem.*, **58,** 541–548.

STEIN, J.M. & MARTIN, B.R. (1984). The effect of carbacyclin, a prostaglandin analogue on adenylate cyclase activity in platelet membranes. *FEBS Lett.*, **165,** 290–292.

TATESON, J.E., MONCADA, S. & VANE, J.R. (1977). Effects of prostacyclin (PGX) on cyclic AMP concentrations in human platelets. *Prostaglandins*, **13,** 389–396.

TREMOLI, E., MADERNA, P., SIRTORI, M. & SIRTORI, C.R. (1979). Platelet aggregation and malondialdehyde formation in type IIA hypercholesterolemic patients. *Haemostasis*, **8,** 47–53.

WHITTLE, B.J.R. & MONCADA, S. (1984). Antithrombotic assessment and clinical potential of prostacyclin analogues. In *Progress in Medicinal Chemistry*, Volume 21. ed. Ellis G.P. & West G.B. pp. 238–279. Amsterdam: Elsevier Science Publishers B.V.

WHITTLE, B.J.R., MONCADA, S., WHITING, F. & VANE, J.R. (1980). Carbacyclin–a potent stable prostacyclin analogue for the inhibition of platelet aggregation. *Prostaglandins*, **19,** 605–627.

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