Estimated Rate of Prostacyclin Secretion into the Circulation of Normal Man

GARRET A. FITZGERALD, ALAN R. BRASH, PIERRE FALARDEAU, and JOHN A. OATES,
Division of Clinical Pharmacology, Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

ABSTRACT The rate of secretion of prostacyclin (PGI_2) into the circulation of normal man was estimated by measurement of the 2,3-dinor-6-keto-$PGF_{1\alpha}$ (D) and 15-keto-13,14-dihydro-2,3-dinor-6-keto-$PGF_{1\alpha}$ (KDD) urinary metabolites of PGI_2. Subjects received 6-h intravenous infusions of vehicle alone and PGI_2 at 0.1, 0.4, and 2.0 ng/kg per min in random order. The fractional elimination of the metabolites was independent of the rate of PGI_2 infusion. 6.8±0.3% of the infused PGI_2 appeared as D and 4.1±0.4% as KDD. The regression of infused PGI_2 upon the quantities of the two metabolites excreted in excess of control values permitted estimation of the rate of entry of endogenous PGI_2 into the circulation corresponding to a given quantity of metabolite excreted. Using the quantities excreted in the 24 h from commencement of the infusions the estimated rates were 0.08±0.02 ng/kg per min from D and 0.10±0.03 from KDD. Studies with exogenous PGI_2 suggest that infusion rates of 2–4 ng/kg per min are required to achieve the threshold for inhibition of platelet function (ex vivo) in man. Although not precluding a role for PGI_2 in local platelet-vessel wall interactions, the much lower estimates obtained in this study suggest that endogenous PGI_2 is unlikely to act as a circulating antiplatelet agent in healthy man.

INTRODUCTION

Prostacyclin (PGI_2)[1] relaxes vascular smooth muscle (1) and potently inhibits platelet aggregation (2). Unlike other prostaglandins (3), PGI_2 is not extensively metabolized by the lung (4) and has been proposed as a physiologically important hormone in vivo. Both Gryglewski et al. (5) and Moncada et al. (6) demonstrated that PGI_2 binding antibodies cause a decrease in platelet aggregate deposition on superfused ex vivo tendons (7) in anaesthesized rabbits and cats. This effect was more marked in arterial than venous blood, suggesting the pulmonary generation of a systemically active, PGI_2-like substance. However, other investigators using PGI_2-binding antibodies failed to obtain evidence in support of this concept (8–10). The present investigation was designed to estimate the rate of entry of endogenous PGI_2 into the circulation in man. The results obtained suggest that PGI_2 is not a physiologically important circulating antiplatelet hormone in normal man.

METHODS

Subjects and study design. Four male volunteers aged 25–45 yr were investigated. Their weight ranged from 68 to 76 kg and they abstained from all medication for 8 wk before the study. All were Caucasian and nonsmokers. The protocol was approved by the Committee for the Protection of Human Subjects of Vanderbilt Medical School.

The subjects received 6-h intravenous infusions of vehicle alone (glycine buffer, pH 10.5), and PGI_2 at 0.1, 0.4, and 2.0 ng/kg per min in random order. Low rates of infusion were chosen to minimize the likelihood of altering metabolism secondary to changes in organ-related blood flow. Infusions were separated by 6-d intervals. Urine was collected for determination of PGI_2 metabolites in two hourly aliquots during and immediately after the infusion. The subjects were permitted a light breakfast on the morning of the investigation. The studies commenced at 1400 h and a snack was provided at 1700 h. A collection of urine for PGI_2 metabolites was obtained on a separate occasion during which the volunteers were ambulant.

Analytical methods. The rate of entry of endogenous prostacyclin into the circulation was estimated by quantification of the 2,3-dinor-6-keto-$PGF_{1\alpha}$ and the 15-keto-13,14-dihydro-2,3-dinor-6-keto $PGF_{1\alpha}$ metabolites of PGI_2 in urine. Stable isotope ratio methods were developed, using gas-chromatography-mass spectrometry in the selected ion monitoring mode (11). Briefly, the initial step was synthesis of deuterium- and tritium-labeled 6-keto-$PGF_{1\alpha}$, which subsequently was converted to the metabolites. Deuterium was incorporated into positions 10 and 8 of PGE_2 by exchange with deuterium-labeled carbitol, and the 9-ketone reduced to yield deuterated $PGF_{2\alpha}$. This was derivatized to

Dr. Falardeau's present address is Institut de Recherche Clinique de Montreal, Quebec.

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[1] *Abbreviation used in this paper*: PGI_2, prostacyclin.

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the methyl ester and converted via the iodo-ether to (8,10,10-$^2H_3$)-$PGF_{2\alpha}$. In a similar procedure, tritium was incorporated into the 9 position by the reduction of the deuterium-labeled PGE_2 by sodium borotritiide. Recovery and identification of the metabolites was monitored by addition of deuterium- and tritium-labeled metabolites of 6-keto-$PGF_{1\alpha}$ to the urine before extraction, derivatization and gas-chromatography-mass spectrometry.

Statistical analysis. Significance of the data was evaluated by Student's t test. Owing to the inequality of variance in the metabolites excreted at different doses of PGI_2, regression analysis was performed by a weighted least squares approach (12). Two-tailed probabilities were employed throughout the analysis. All values are expressed as the mean±SEM.

RESULTS

Blood pressure and heart rate were unchanged from control values during the PGI_2 infusions. There was no significant difference between the total quantities of 2,3-dinor-6-keto-$PGF_{1\alpha}$ (499.9±46 ng vs. 374.9±80.2 ng; $P > 0.10$) and 15-keto-13,14-dihydro-2,3-dinor6-keto-$PGF_{1\alpha}$ (229.1±33 ng vs. 200.0±49 ng; $P > 0.10$) excreted on the ambulant and infusion control days. The rates of excretion of the two metabolites during the collection periods on the infusion control day are illustrated in Table I. Metabolite excretion increased during the sequential 2-h collection periods within each PGI_2 infusion (Fig. 1). The fractional elimination of the metabolites was independent of the rate of PGI_2 infusion. A mean 6.8% of the infused PGI_2 appeared as 2,3-dinor-6-keto-$PGF_{1\alpha}$ and a mean 4.1% as 15-keto-13,14-dihydro-2,3-dinor-6-keto-$PGF_{1\alpha}$ (Table II).

Interpolation of metabolite values obtained on the control infusion day onto the linear relationship between the quantities of infused PGI_2 and the amount of the metabolite excreted in excess of control values permitted calculation of the rate of entry of endogenous PGI_2 into the circulation (Fig. 2). For these purposes, it was assumed that the linear relationship between the rate of PGI_2 infused and the quantities of metabolites excreted existed for PGI_2 below 0.1 kg/min. Although it would be logically expected that when the Y variable (infused PGI_2) was zero the X variable (quantity of urinary metabolite excreted in excess of control values) would also be zero, the regression lines were tested against the hypothesis that the Y intercept was zero prior to forcing the lines through the origin (13). The regressions of infused PGI_2 upon both the 2,3-dinor-6-keto-$PGF_{1\alpha}$ ($t = 1.46$; $P > 0.05$) and the 15-keto-13,14-dihydro-2,3-dinor-6-keto-$PGF_{1\alpha}$ ($t = 0.047$; $P > 0.05$) yielded estimates of the Y intercept which were not significantly different from zero.

Linear equations were obtained by regression of infused PGI_2 upon the 24-h quantities of the metabolites excreted in excess of control values. Insertion of the quantities of the metabolites excreted during the 6-h control infusions into these equations permitted estimation of the rate of entry of endogenous PGI_2 into the circulation. The estimated rates were 0.08±0.02 ng/kg per min from the 2,3-dinor-6-keto-$PGF_{1\alpha}$ metabolite and 0.10±0.03 ng/kg per min from 15-keto-13,14-dihydro-2,3-dinor-6-keto-$PGF_{1\alpha}$ (Table III).

TABLE I
Urinary Metabolites of Prostacyclin: Control Values

	2,3-dinor-6-keto prostaglandin $F_{1\alpha}$	15-keto-13,14-dihydro-2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$
	pg/mg creatinine	*pg/mg creatinine*
Ambulant control, 24 h	234.4±37.1	138.4±36.5
Control infusion		
0–2 h	243.2±55.1	182.5±33.7
2–4 h	196.2±29.7	163.3±54.8
4–6 h	266.7±68.7	246.7±88.5
6–18 h	282.5±23.9	93±39.4
18–24 h	314.9±106.8	72.4±18.1

The excretion of the prostacyclin metabolites is expressed per milligram of creatinine excreted during the two control periods: (*a*) "ambulant" control, when a 24-h collection was performed while the subjects were otherwise unrestricted (*b*) infusion control, when the subjects received an intravenous infusion of vehicle alone (glycine buffer pH 10.5) from hours 0–6, during which they lay supine and were thereafter unrestricted.

Finally, these metabolites arise by enzymatic conversion from PGI_2 or 6-keto-$PGF_{1\alpha}$ (14). The major portion of this enzymatic activity is thought to occur in the liver and kidney following the entry of PGI_2 into the circulation. However, should some conversion occur within the organ of biosynthesis the value that we have obtained may slightly overestimate the rate of PGI_2 secretion into the bloodstream.

DISCUSSION

We have estimated the rate of entry of endogenous PGI_2 into the bloodstream by quantification of 2 major urinary metabolites of PGI_2 in man (14, 15). Because PGI_2 may have been converted in part to these metabolites prior to its entry into the circulation, the rates obtained represent maximal estimates. The mean value obtained using the 2,3-dinor-6-keto-$PGF_{1\alpha}$ metabolite (0.08 ng/kg per min) was remarkably concordant with the estimate derived from the 15-keto-13,14-dihydro-2,3-dinor-6-keto-$PGF_{1\alpha}$ (0.10 ng/kg per min).

Studies with exogenous PGI_2 suggest that infusion rates of 2–3 ng/kg per min are required to achieve the threshold for either inhibition of platelet function ex vivo (16) or systemic hemodynamic effects in man (16, 17). Although the threshold antiplatelet effect of prostacyclin in vivo may be somewhat less than studies ex vivo suggest, the much lower estimates of the rate of

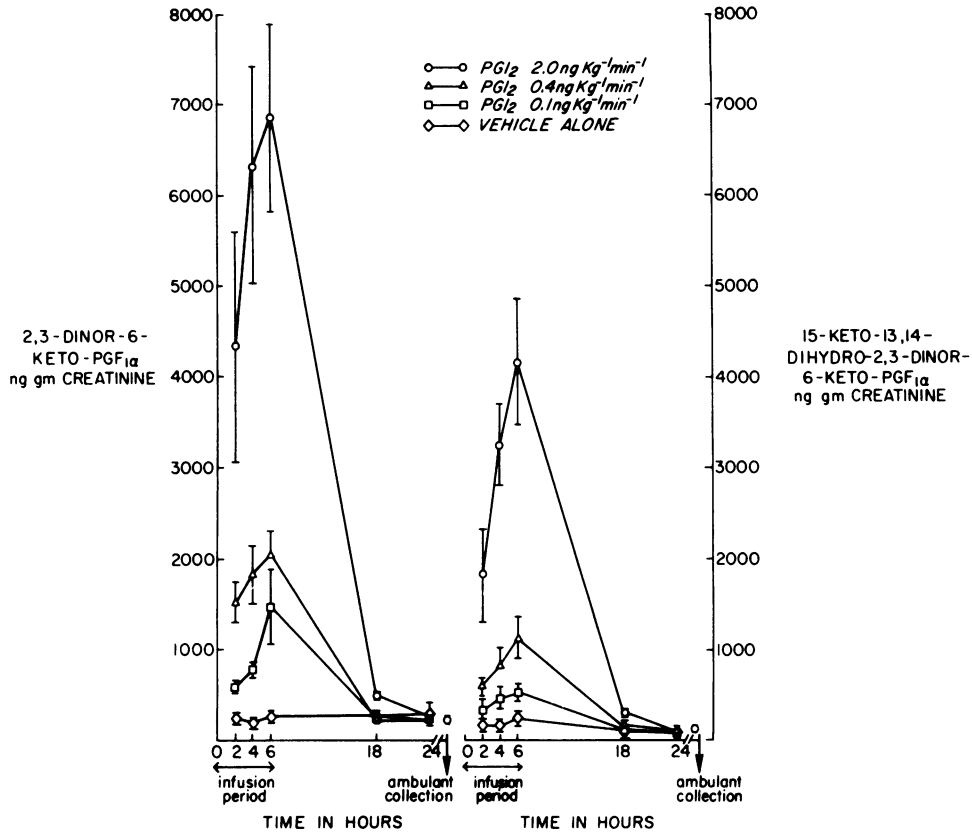

FIGURE 1 Changes in the rate of excretion of 2,3-dinor-6-keto-$PGF_{1\alpha}$ and 15-keto-13,14-dihydro-2,3-dinor-6-keto-$PGF_{1\alpha}$ during and after 6-h infusions of PGI_2 and vehicle (glycine buffer pH 10.5) alone. The quantity of the metabolite excreted during an unrestricted 24-h collection (ambulant control) is also shown.

entry of endogenous PGI_2 into the bloodstream suggest that it is unlikely to have a physiological role as a circulating hormone in man.

The suggestion that the lungs might act, not only as filters for unwanted vasoactive substances, but as generators of circulating hormones was supported by the observations of Gryglewski (5) and Moncada (6) and their colleagues. They demonstrated that exogenous PGI_2 decreased superfused ex vivo tendon weight in heparinized, anesthetized rabbits and cats and that this effect was inhibited by infusion of an antiserum to a stable PGI_2 analogue (5,6-dihydro-PGI_2). Tendon weight gain, due to deposition of platelet aggregates (17) occurred in the absence of exogenous PGI_2 and was enhanced by the additional PGI_2 antibodies. This effect was more pronounced in arterial than venous blood, suggesting the pulmonary generation of a systemically active, PGI_2-like substance. This proposal was further supported by the demonstration of an arterio-venous gradient of 6-keto-$PGF_{1\alpha_1}$, the hydration production of PGI_2, in humans undergoing cardiac catheterization (18).

However, Smith et al. (8) failed to demonstrate an effect of infusion of PGI_2-binding antibodies (to 6-keto-$PGF_{1\alpha}$) on basal blood pressure in anesthetized cats even though the antiplatelet and vasodepressor response to a concomitant infusion of exogenous PGI_2 could be inhibited. Steer et al. (9) found that addition of

TABLE II

Percentage Conversion of Prostacyclin to Urinary Metabolites

	24-h period from start of infusion	
Rate of PGI_2 infusion	2,3-dinor-6-keto prostaglandin $F_{1\alpha}$	15-keto-13,14-dihydro-2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$
ng/kg per min	%	%
0.1	7.5±0.6	4.0±1.2
0.4	6.6±1.4	4.2±0.4
2.0	6.0±1.0	2.9±0.6
Mean of 3 infusions	6.8±0.3	4.1±0.4

The fractional conversion rate of infused PGI_2 into the two metabolites is expressed for the 24-h period commencing with each infusion. Fractional conversion rates are also expressed for each individual's mean over the three rates of PGI_2 infusion.

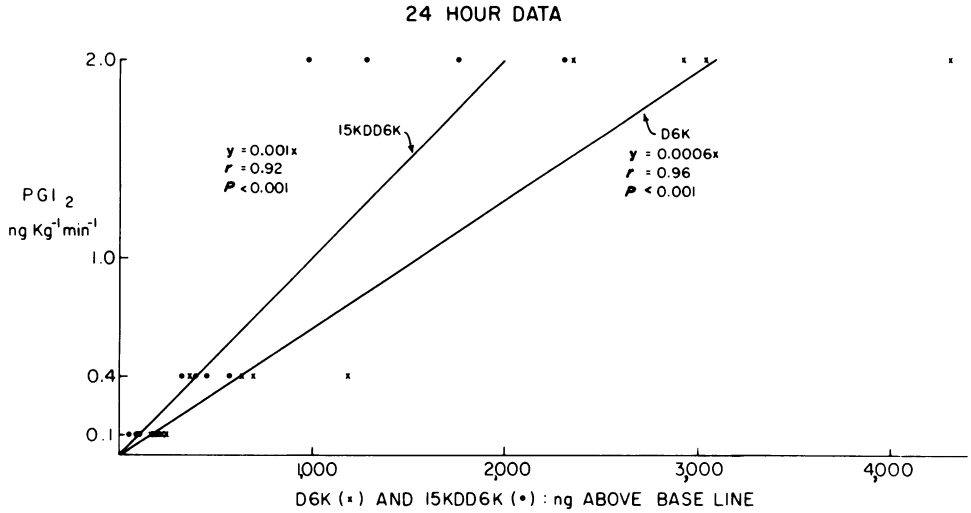

FIGURE 2 Infused PGI_2 is regressed upon the quantities of two urinary metabolites, 2,3-dinor-6-keto-$PGF_{1\alpha}$ (D6K) and 15-keto-13,14-dihydro-2,3-dinor-6-keto-$PGF_{1\alpha}$ (15 KDD6K) excreted in excess of control values during the infusion of vehicle (glycine buffer, pH 10.5) alone in four subjects. Insertion of the 6-h (corresponding to the duration of infusion) control value of D6K or 15 KDD6K into the appropriate linear equation permits estimation of the rate of entry of endogenous PGI_2 into the circulation (Table III). Having established that the Y-intercept did not differ significantly from zero, the linear regressions were constrained to pass through the origin.

PGI_2 binding antibodies (to 5,6-dihydro-PGI_2) to human platelet-rich plasma after either arterial or venous blood collection failed to alter platelet aggregation. Addition of these antibodies to platelet-rich plasma inhibited the effects of exogenous PGI_2 on both aggregation and platelet cyclic AMP. Whereas these investigations suggested that PGI_2 was unlikely to act as a physiologically important antiplatelet circulating agent, similar studies with antibodies to 5,6-dihydro-PGI_2 led Pace-Asiak and his colleagues (10) to conclude that PGI_2 did not act as a systemic vasodepressor hormone in the normotensive or hypertensive rat.

Considerable controversy also exists as to the concentration of 6-keto-$PGF_{1\alpha}$ in human plasma. Estimates have ranged from 10–15 ng/ml using superfusion techniques (19) to 100–200 pg/ml using both radioimmunoassay (20) and gas chromatography-mass spectrometry (21). Recently the use of gas chromatograph-electron capture methods suggests that "resting" levels may be much lower (22).

Myatt et al. (23) estimated the clearance rate of PGI_2 in man by infusing exogenous PGI_2 and measuring 6-keto-$PGF_{1\alpha}$ at pseudo-steady state. Because of concentrations of 6-keto-$PGF_{1\alpha}$ measured in the bloodstream represent a mixture of PGI_2 and 6-keto-$PGF_{1\alpha}$, the clearance of 26.2±4.7 ml/kg per min that they obtained would tend to underestimate total plasma clearance of endogenous PGI_2. Thus, a maximal estimate of the circulating concentration of PGI_2 may be obtained by dividing the rate of entry of PGI_2 into the bloodstream (~0.09 ng/kg per min) by this rate of plasma clearance (~26.2 ml/kg per min) resulting in 3.4 pg/ml. This is an order of magnitude less than the

TABLE III
Estimated Rate of Entry of Endogenous PGI_2 into the Circulation

2,3-dinor-6-keto-$PGF_{1\alpha}$ (D6K):

	Control excretion of D6K, *ng*	Entry rate of PGI_2, *ng/kg per min*
Subjects		
1	182.8	0.11
2	114.3	0.07
3	72.1	0.04
4	173.1	0.10
Mean±SEM	135±26.0	0.08±0.02

15-keto-13,14-dihydro-2,3-dinor-6-keto-$PGF_{1\alpha}$ (15KDD6K):

	Control excretion of 15KDD6K, *ng*	Entry rate of PGI_2, *ng/kg per min*
Subjects		
1	74.0	0.07
2	173.7	0.17
3	116.1	0.12
4	52.1	0.05
Mean±SEM	104.0±26.8	0.10±0.03

Total quantities of the two metabolites excreted on the "infusion" control day are expressed for the 6-h infusion period. The regression of the PGI_2 infusion rate onto the quantities of the two metabolites excreted in excess of control values in the 24 h from the start of the infusion permit estimation of the rate of entry of endogenous PGI_2 into the circulation for a given metabolite excretion (Fig. 2).

threshold concentration of PGI_2 required (9) to inhibit aggregation in vitro of platelets obtained from human volunteers (36 pg/ml). The threshold for patients with peripheral vascular disease has been reported (24) to be much higher (1,500 pg/ml).

In conclusion, we have measured the excretion of two urinary PGI_2 metabolites during the infusion of exogenous PGI_2 over a 20-fold dose range to enable estimation of the rate of entry of endogenous PGI_2 into the bloodstream. Our results do not preclude an important role for PGI_2 in the local regulation of platelet vessel wall interaction. However they do suggest that secretion from the lungs or other organs, and subsequent systemic circulation of PGI_2 does not represent an important homeostatic mechanism in vivo.

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