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Effects of intravenous administration of prostacyclin on regional blood circulation in awake rats

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1 The effects of intravenous infusion of prostacyclin (PGI_2, 0.1, 0.2, 0.3 and 1.0 $\mu g\ kg^{-1}\ min^{-1}$ lasting 5 min) on regional blood flow and regional vascular resistance have been studied in awake rats using the radioactive microsphere method.

2 The control values of blood flow to the heart, kidney, small intestine, hind limb muscle, pericranial skin and brain as well as the corresponding vascular resistance were not modified by an i.v. infusion (0.1 ml min^{-1}) of Tris-buffer (the vehicle of PGI_2).

3 The i.v. infusion of PGI_2 produced graded dose-dependent decreases of MAP ($r=0.87$, $P<0.001$; $ED_{20}=0.73$ [0.13–2.55] $\mu g\ kg^{-1}\ min^{-1}$) as well as decreases of vascular resistance in the heart ($r=0.83$, $P<0.001$; $ED_{30}=0.17$ [0.09–0.31] $\mu g\ kg^{-1}\ min^{-1}$), pericranial skin ($r=0.88$, $P<0.001$; $ED_{30}=0.28$ [0.18–0.43] $\mu g\ kg^{-1}\ min^{-1}$) and small intestine ($r=0.74$, $P<0.001$; $ED_{30}=0.21$ [0.11–0.39] $\mu g\ kg^{-1}\ min^{-1}$), which led to dose-related increases of blood flow to these territories.

4 On the contrary, PGI_2 increased vascular resistance in skeletal muscle ($r=0.73$, $P<0.001$; $ED_{30}=0.20$ [0.10–0.39] $\mu g\ kg^{-1}\ min^{-1}$) with corresponding reductions in blood flow. The low doses reduced renal blood flow but there were no significant changes during the high ones. Cerebral vessels did not dilate during any infusion of PGI_2 and cerebral blood flow decreased as MAP fell ($r=0.56$, $P<0.01$).

5 We conclude that, in awake rats, the coronary vessels are extremely sensitive to the vasodilating effect of PGI_2 and that the mesenteric vessels and those of the pericranial skin are very responsive too. Moreover, autoregulation is inefficient to maintain cerebral blood flow during infusion of PGI_2.

Keywords: Prostacyclin; blood flow; vascular resistance; radioactive microspheres; coronary circulation; cerebral circulation; renal circulation; mesenteric circulation; pericranial skin vessels; skeletal muscle blood flow

Abbreviations: MAP, mean arterial blood pressure; PGI_2, prostacyclin

Introduction

The discovery that arterial walls generated PGI_2 (Moncada *et al*., 1976) which relaxed strips of mesenteric and celiac arteries of rabbits (Bunting *et al*., 1976), focused the attention of many scientists on this prostaglandin. It was verified that PGI_2 had a dilator activity on various vascular beds in different animal species and humans (Moncada & Vane, 1979), and that, unlike other prostaglandins, PGI_2 is equipotent as hypotensive after its i.v. or i.a. administration because it is not inactivated during its passage through the lungs (Armstrong *et al*., 1978). These findings led to the proposition that this prostaglandin is a potent endogenous vasodilating substance that actively participates in the regulation of blood pressure and circulation (Armstrong *et al*., 1978; Moncada & Vane, 1979).

However, it was demonstrated that PGI_2 is not a circulating hormone (Blair *et al*., 1982), and it was suggested that, in certain blood vessels, prostaglandins other than PGI_2 are important endogenous modulators of contractility (Förstermann *et al*., 1984). Thus, prostaglandin E_2 production increases relative to PGI_2 as vessel diameter decreases (Gerritsen, 1987). There is regional variability in PGI_2 production with greater activity in the more proximal or centrally located vessels (Fann *et al*., 1989) and PGI_2 must be considered a local hormone rather than a circulating one (Vane *et al*., 1990). Moreover, some blood vessels do not dilate in response to PGI_2 (Frölich & Förstermann, 1989), and many of the evidences for physiological or pathophysiological contribution of PGI_2 as a vasodilating substance are indirect ones, that is, they are the result of vascular modifications induced by indomethacin, which has a vasoconstrictor activity unrelated with inhibition of cyclo-oxygenase (Quintana *et al*., 1988). Consequently, the true role of PGI_2 in the regulation of regional blood circulations as well as the effectiveness of exogenous PGI_2 to enhance perfusion in compromized areas may depend on both animal species and vascular bed considered.

In spite of the large number of reports on the vascular effects of PGI_2, only one has studied the effect of exogenous PGI_2 on various regional blood circulations simultaneously (Smith *et al*., 1978). In this work, the blood flow to different organs and tissues (the brain is not included) was measured in control anaesthetized cats and compared with that measured in animals receiving an i.v. infusion of PGI_2. In rats, Steinberg *et al*. (1988) evaluated the effects of i.v. infusion of different doses of a PGI_2 derivative (iloprost) on renal, mesenteric and hind limb blood flows, although actual values of flow (ml min^{-1}) were not measured.

The intravenous infusion of PGI_2 or iloprost (stable analogue) has been used in the therapy of severe peripheral vascular disease and pulmonary hypertension (Grant & Goa, 1992; Wise & Jones, 1996). Recently, it has been demonstrated that continuous intravenous infusion of prostacyclin improved survival in patients with severe primary pulmonary hypertension, as compared with conventional therapy (Barst *et al*.,

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1996) and it is also effective in newborns (Eronen *et al*., 1997). Moreover, it has been proposed for the treatment of heart failure after cardiac surgery (Kieler-Jensen *et al*., 1995). Consequently, better knowledge of the effects produced by the i.v. infusion of prostacyclin on the regional blood circulations may be helpful to extend and/or improve the therapeutic applications of this prostaglandin.

In the present study we have determined blood flow and vascular resistance in various organs and tissues of awake rats as well as the effect of i.v. PGI_2 infusion and we have analysed the dose-response relationship for PGI_2 in each vascular bed studied, including heart, kidney, skeletal muscle, small intestine, pericranial skin and the brain.

Methods

Male Sprague-Dawley rats (300–320 g) from our own facilities were under constant environmental conditions (22–24°C; relative humidity 55%; 12 h light/dark periods). Animals were used throughout the study according to the European Convention for the Protection of Vertebrate Animals Used for Research and other Scientific Purposes.

Determination of regional blood flow and regional vascular resistance

The rats were surgically prepared the day before the experiment as described previously (Quintana *et al*., 1983). Rats were anaesthetized with sodium pentobarbital (40 mg kg^{-1}, i.p.) and polyethylene catheters (Becton Dickinson) were introduced and fixed into the left ventricle (PE10, *via* right carotid artery), into the abdominal aorta (PE50, *via* left femoral artery), and into the superior caval vein (PE10, *via* right jugular vein). These catheters were filled with heparinized saline solution (100 iu ml^{-1}), sealed and exteriorized in the upper part of the neck. The animals were housed in individual boxes with free access to water and chow. Next day, each rat was placed in a small opaque box the front part of which was closed with a metal net; this allowed us to perform drug and microsphere administrations without disturbing the animals. All the experiments were performed at room temperature (21–24°C). The reference sample radioactive microsphere method (Bonaccorsi *et al*., 1978) was applied to obtain two determinations of regional blood flow in each rat. For each blood flow determination, about 120,000–140,000 microspheres (Du Pont de Nemours) 16.5 ± 0.1 μm in diameter, labelled either with cerium-141 (^{141}Ce, 3.12 mCi g^{-1}) or ruthenium-103 (^{103}Ru, 9.58 mCi g^{-1}) and suspended in 0.4 ml of rat serum were injected into the left ventricle over a 20 s period. Withdrawal of the reference blood sample (through the femoral catheter) at a rate of 0.375 ml min^{-1} began 10 s prior to microsphere injection and continued for 90 s afterwards. Blood was collected in a polyethylene tubing (PE 205) coupled to a glass syringe mounted in an infusion pump (B. Braun). Immediately before microsphere injection, the partial pressures of oxygen (PO_2) and carbon dioxide (PCO_2), as well as the pH were determined in an arterial blood sample of 50 μl (collected in a heparinized glass capillary tube) by using an automatic blood gas analyzer (AVL 940). The MAP was monitored (except when blood was withdrawn) through the femoral catheter by using a Statham P23Db transducer connected to a Beckman R411 dynograph which recorded the electronically damped mean signal. Injection of microspheres and withdrawal of the blood sample in the way described has no effect on MAP in the awake rat and the values registered both immediately before and after completion of the reference blood withdrawal never differed by more than 4 mmHg. The mean of those values was taken as the corresponding value of MAP for that microsphere injection.

After completion of the second microsphere injection, the rats were anaesthetized (sodium pentobarbital, 30 mg kg^{-1} i.v.) then killed with an i.v. injection of a KCl-saturated solution. Since microspheres are trapped in the first passage through the small vessels of the tissues, this way of sacrifice of the animals does not interfere with flow measurements (Bonaccorsi *et al*., 1978; Flaim *et al*., 1984). The heart, kidneys, lungs, brain, small intestine, the skin over the head and the right cruralis hind limb muscle were carefully removed, weighed and placed in counting vials for radioactivity measurements in a well-type scintillation gamma counter (Packard Autogamma 5650). The radioactivity in the reference blood samples was also measured and blood flow (ml g^{-1} min^{-1}) to each organ or tissue was calculated as previously described (Bonaccorsi *et al*., 1978). The regional vascular resistance (mmHg ml^{-1} g min^{-1}) was calculated as the MAP (mmHg) divided by the corresponding blood flow (ml g^{-1} min^{-1}).

Experimental protocols

The first microsphere injection was performed in each rat 5 min after the beginning of an i.v. infusion (0.1 ml min^{-1}) of Tris buffer (0.05 M Tris-HCl buffer, pH 8); this was the vehicle used for PGI_2 administration. The infusion finished when microsphere injection and blood withdrawal were completed.

The second microsphere injection was performed approximately 60 min later. The animals (six per group) were assigned to one of the following experimental groups: (1) Control vehicle which received a Tris buffer i.v. infusion (as described above); (2) PGI_2 0.1 μg kg^{-1} min^{-1} i.v.; (3) PGI_2 0.2 μg kg^{-1} min^{-1} i.v.; (4) PGI_2 0.3 μg kg^{-1} min^{-1} i.v.; (5) PGI_2 1.0 μg kg^{-1} min^{-1} i.v. All the infusions finished when blood withdrawal was completed. Consequently, infusions lasted 7 min.

The PGI_2 solutions for infusion were prepared immediately prior to its administration and maintained cold (1–2°C). The infusions were performed with the aid of a constant peristaltic pump (LKB 2120 Varioperpex) covered with a plastic bag containing ice. In this way, the time that PGI_2 solutions were at room temperature was negligible. Since the half life of PGI_2 at pH 8 and 25°C is approximately 25 min (Cho & Allen, 1978), the actual values of PGI_2 administered should be close to the nominal ones.

Data presentation and statistical analysis of the results

Since vascular resistance is a ratio and as such it is not normally distributed, the experimental values (mmHg min^{-1} g ml^{-1}) were transformed to the corresponding natural logarithms prior to statistical analysis. The mean, s.e.mean and the 95% confidence limits of the mean were obtained in the usual way on the ln scale, and then converted back to the original scale. Consequently, geometric mean and asymetric s.e.mean or 95% confidence limits are obtained (Armitage & Berry, 1987). The effects of PGI_2 on regional vascular resistance have been expressed as the mean percentage change regarding the corresponding control value $\pm$ s.e.mean. Blood flow was expressed in absolute values (ml g^{-1} min^{-1}) as the mean $\pm$ s.e.mean. The values of MAP, and oxygen and carbon dioxide tension in arterial blood have been expressed in mmHg and the mean $\pm$ s.e.mean for each experimental group and condition calculated.

Comparison between control (first microsphere injection) and treatment (second microsphere injection) values obtained in each experimental group was made using the two tailed paired Student *t*-test. Correlations between PGI_2 log dose and effect on both regional blood flow and vascular resistance have been analysed. Where a significant correlation was detected, the corresponding ED_{30}, that is, the dose which induces a 30% change regarding the control was calculated, as well as its 95% confidence limits which are given within brackets. Since the slope of the regression lines corresponding to MAP and cerebral blood flow were low, the ED_{20} instead of the ED_{30} have been calculated for these effects. The correlation between changes in MAP and cerebral blood flow during PGI_2 infusion has been studied too. All the statistical analyses were performed with the aid of a computer program (StatView 4.0).

Drugs and reagents

The following substances were used: PGI_2 (sodium salt) and Tris (hydroxymethyl) aminomethane (Tris buffer 0.1 M solution pH 9.0 at 25°C and Tris-HCl 0.05 M pH 8.0 at 25°C) from Sigma; hydrogen chloride, potassium chloride and sodium chloride from E. Merck; sodium pentobarbital (Nembutal, Abbott); sodium heparin (Heparina Rovi 5%).

Physiological saline (9.0 g l^{-1} NaCl in distilled deionized water) was prepared weekly and maintained at 4–5°C. Sodium heparin (100 iu ml^{-1} in physiological saline) was prepared daily, as well as sodium pentobarbital (20 mg ml^{-1} in distilled water). A saturated solution of potassium chloride in distilled water was prepared weekly and maintained at room temperature. The Tris-HCl 0.05 M was prepared daily in a 0.6% NaCl to give an isotonic solution that was maintained cold (1–2°C) with ice. A stock solution (1 mg ml^{-1}) of PGI_2 was prepared in 0.1 M Tris buffer solution pH 9 and divided in 100 μl aliquots stored at −80°C for weekly use; immediately prior to use, an appropriate PGI_2 solution (approximately 0.3, 0.6, 0.9, and 3.0 μg ml^{-1}) was prepared in cold (1–2°C) 0.05 M Tris-HCl saline solution pH 8, to administrate the corresponding dose at a rate of 0.1 ml min^{-1}. Since values of buffer pH are temperature-dependent, the actual pH of the solutions administered should be slightly higher.

Results

In the control group of rats, the values of blood flow to the heart, kidney, brain, small intestine, pericranial skin and hind limb muscle, obtained by the first microsphere injection, were close to the corresponding values determined 1 h later by the second injection of microspheres (Table 1). Since the MAP was unchanged (as shown in Table 2), regional vascular resistances were not modified (Table 1). Arterial blood pH, PO_2 and PCO_2 were unchanged too (Table 2).

In contrast, graded dose-dependent decreases of MAP ($r = 0.85$, $P < 0.001$) were induced by the i.v. infusion of PGI_2 (Table 2). The ED_{20} calculated from the regression line was

Table 1 Regional blood flow and vascular resistance in the control group of rats. There was a 60 min interval between first and second determinations

	First determination		*Second determination*	
Organ or tissue	*Flow** (ml g^{-1} min^{-1})	*Vascular resistance†* (mmHg g min ml^{-1})	*Flow** (ml g^{-1} min^{-1})	*Vascular resistance†* (mmHg g min ml^{-1})
Heart	6.61±0.70	17.7 (13.2–24.5)	6.80±0.81	17.1 (12.9–23.9)
Kidney	5.22±0.41	22.2 (17.8–27.4)	5.03±0.33	22.5 (18.4–27.4)
Brain	1.11±0.09	104 (89–120)	1.09±0.07	105 (93–119)
Pericranial skin	0.29±0.02	392 (315–487)	0.27±0.03	421 (331–535)
Limb muscle	0.25±0.04	469 (363–604)	0.24±0.03	480 (381–603)
Small intestine	2.45±0.19	47.4 (37.6–59.6)	2.39±0.17	47.9 (38.8–59.1)

*Mean values±s.e.mean determined in six rats. †Mean values and their 95% confidence limits within brackets determined in six rats. The values were obtained through microspheres injections performed after 5 min the beginning of an i.v. infusion of 0.1 ml min^{-1} 0.05 M Tris-HCl buffer in saline solution, pH 8.

Table 2 MAP and arterial blood PO_2, PCO_2 and pH determined in the various groups of rats immediately before the microspheres injections

Experimental group and condition	*MAP* (mmHg)	*pH*	*PO_2* (mmHg)	*PCO_2* (mmHg)
Control				
Tris-HCl	115±2	7.41±0.01	92.5±1.2	36.1±1.0
Tris-HCl	114±3	7.41±0.01	93.3±1.4	35.8±1.1
Prostacyclin 0.1 μg kg^{-1} min^{-1}				
Tris-HCl	116±3	7.41±0.01	93.4±0.9	35.6±1.0
Prostacyclin	112±3*	7.41±0.01	97.1±2.1	34.7±0.8
Prostacyclin 0.2 μg kg^{-1} min^{-1}				
Tris-HCl	119±2	7.41±0.01	94.2±1.0	36.3±1.3
Prostacyclin	113±1†	7.41±0.01	97.5±1.7	35.1±1.1
Prostacyclin 0.3 μg kg^{-1} min^{-1}				
Tris-HCl	112±2	7.41±0.01	93.8±1.1	35.5±1.2
Prostacyclin	103±3†	7.42±0.01	101.5±2.2*	33.2±1.2
Prostacyclin 1.0 μg kg^{-1} min^{-1}				
Tris-HCl	115±2	7.41±0.01	94.0±1.8	36.1±1.3
Prostacyclin	86±3#	7.42±0.01	106.0±1.2#	31.7±0.9†

*$P < 0.05$, †$P < 0.01$ and #$P < 0.001$ regarding the corresponding Tris-HCl value. Values are the mean±s.e.mean obtained in each group of six animals.

0.73 (0.13–2.55) μg kg^{-1} min^{-1}. In addition, a very slight degree of hyperventilation was produced by the highest dose of PGI_2, as shown by the slight increase of arterial blood PO_2 and the very slight but significant decrease of PCO_2 (Table 2).

As shown in Figure 1, i.v. infusion of PGI_2 produced dose-related increases of blood flow to the heart ($r=0.74$, $P<0.001$) and the calculated ED_{30} was 0.17 (0.12–0.24) μg kg^{-1} min^{-1}; small intestine ($r=0.60$, $P<0.01$), $ED_{30}=0.20$ (0.13–0.31) μg kg^{-1} min^{-1}; and pericranial skin ($r=0.67$, $P<0.001$), $ED_{30}=0.24$ (0.15–0.37) μg kg^{-1} min^{-1}. Nevertheless, the lowest rate of PGI_2 infusion (0.1 μg kg^{-1} min^{-1}) only produced a significant increase of blood flow to the heart and a decrease in that corresponding to the kidney. Blood flows to the hind limb muscle and to the brain decreased by the administration of 0.2, 0.3, and 1.0 μg kg^{-1} min^{-1} of PGI_2. There are significant correlations between the log dose of PGI_2 and the decreases of blood flow to the muscle ($r=0.80$, $P<0.001$), $ED_{30}=0.24$ (0.13–0.44) μg kg^{-1} min^{-1} and brain ($r=0.58$, $P<0.01$), $ED_{20}=0.45$ (0.05–4.5) μg kg^{-1} min^{-1}. There is also a statistically significant correlation between the fall in MAP and the decrease of cerebral blood flow induced by PGI_2 ($r=0.56$, $P<0.01$).

Figure 2 illustrates the changes of vascular resistance induced by PGI_2 in the various organs and tissues studied. As shown, the lowest rate of PGI_2 infusion (0.1 μg kg^{-1} min^{-1}) only dilated the coronary vascular bed and induced a constriction in the renal one which was also constricted by 0.2 μg kg^{-1} min^{-1}. This and the higher doses (0.3 and 1.0 μg kg^{-1} min^{-1}) of PGI_2 produced strong dilation of coronary, mesenteric and pericranial skin vascular beds, while resistance vessels of the hind limb muscle were constricted. Cerebral vessels did not respond to any PGI_2 dose studied. There are significant correlations between the log dose of PGI_2 infused and the decreases of vascular resistance observed in the heart ($r=0.83$, $P<0.001$), $ED_{30}=0.17$ (0.09–0.24) μg kg^{-1} min^{-1}; pericranial skin ($r=0.88$, $P<0.001$), $ED_{30}=0.28$ (0.18–0.43) μg kg^{-1} min^{-1}; and small intestine ($r=0.74$, $P<0.001$), $ED_{30}=0.21$ (0.11–0.39) μg kg^{-1} min^{-1}. In addition, there is also a significant correlation between the log dose of PGI_2 and the rise in vascular resistance in the hind limb muscle ($r=0.73$, $P<0.001$), $ED_{30}=0.20$ (0.10–0.39) μg kg^{-1} min^{-1}.

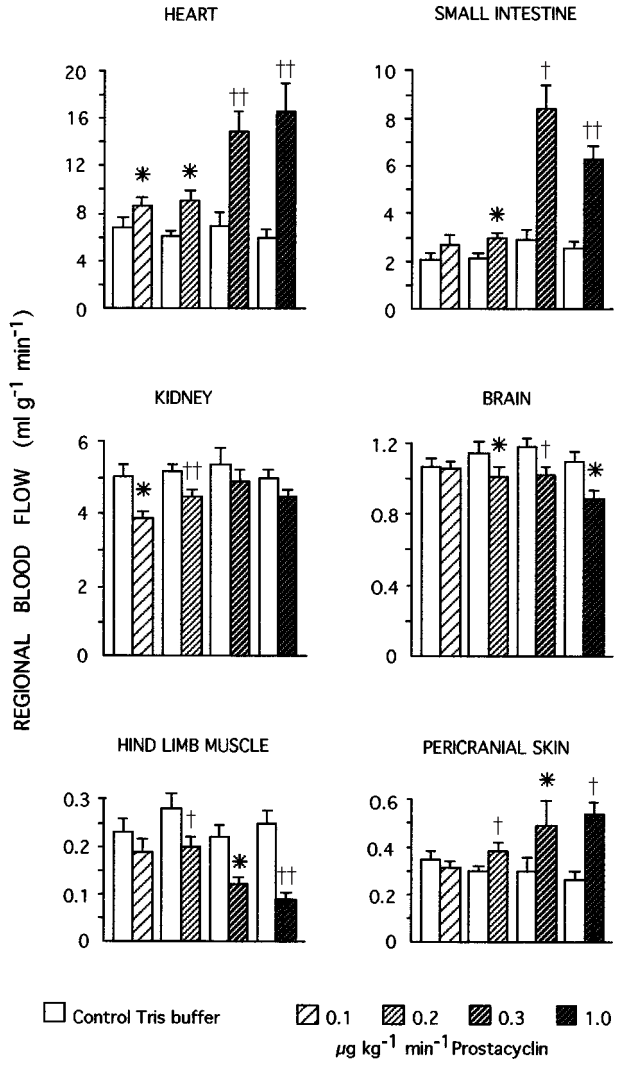

Figure 1 Regional blood flow measured in the experimental groups of rats under control conditions (i.v. infusion of vehicle, 0.1 ml min^{-1}) and during i.v. infusion of prostacyclin (0.1, 0.2, 0.3 and 1.0 μg kg^{-1} min^{-1}). The columns represent the mean values and the bars the s.e.mean of six animals. *$P<0.05$; †$P<0.01$ and ††$P<0.001$ regarding the corresponding control value.

Discussion

This is the first report describing in awake animals the effects of graded doses of PGI_2 on various regional vascular beds simultaneously (including heart, kidney, small intestine, brain, skin and muscle). The control values of MAP found in all the groups studied are similar between them and similar to those reported in awake rats by other authors (Flaim *et al.*, 1984; Hsu *et al.*, 1980; Weeks & Compton, 1979). Moreover, the control values of arterial blood PO_2, PCO_2 and pH are close to that reported previously in awake Sprague-Dawley rats (Quintana *et al.*, 1983). Likewise, control values of regional blood flow in the present work are similar to those reported by other authors using radioactive microspheres in rats, as Flaim *et al.* (1984) for renal, cerebral, coronary and skeletal muscle blood flows; Hsu *et al.* (1980) for renal blood flow; and Lundberg & Smedegård (1981) for skin blood flow.

As expected, PGI_2 produced a hypotensive dose-related effect. As showed by Weeks & Compton (1979), prostacyclin-induced hypotension was significantly lesser in awake than in anaesthetized rats, being the threshold dose in the former 0.1–0.18 μg kg^{-1} min^{-1}. These authors demonstrated that the rise in plasma renin activity induced by PGI_2 in awake animals was responsible for this difference that disappeared after nephrectomy.

The slight elevation of PO_2 and decrease of PCO_2, without modification of blood pH, observed during the infusion of the highest dose of PGI_2 seems the consequence of a quite modest degree of hyperventilation. Although it has been shown that PGI_2 may increase the secretion of bicarbonate by the distal tubule in the bicarbonate-ingesting animals (Wesson, 1996), the author suggests that this prostaglandin does not modulate acidification in the distal tubule under control conditions. Hence, the relatively short duration of the PGI_2 infusion, which was slightly alcaline, seems insufficient to induce a plasma acidification. Some vascular beds, as renal, cutaneous and skeletal muscle show relatively small reactivity to moderately severe changes in PCO_2 and PO_2 (Heistad & Abboud, 1980). It has been shown that a rise in PO_2 from 86 to 362 mmHg decreases skeletal muscle blood flow 22% in rabbits (Thorborg *et al.*, 1990). When rats were ventilated with 100% hyperbaric (two atmospheres) oxygen, renal and

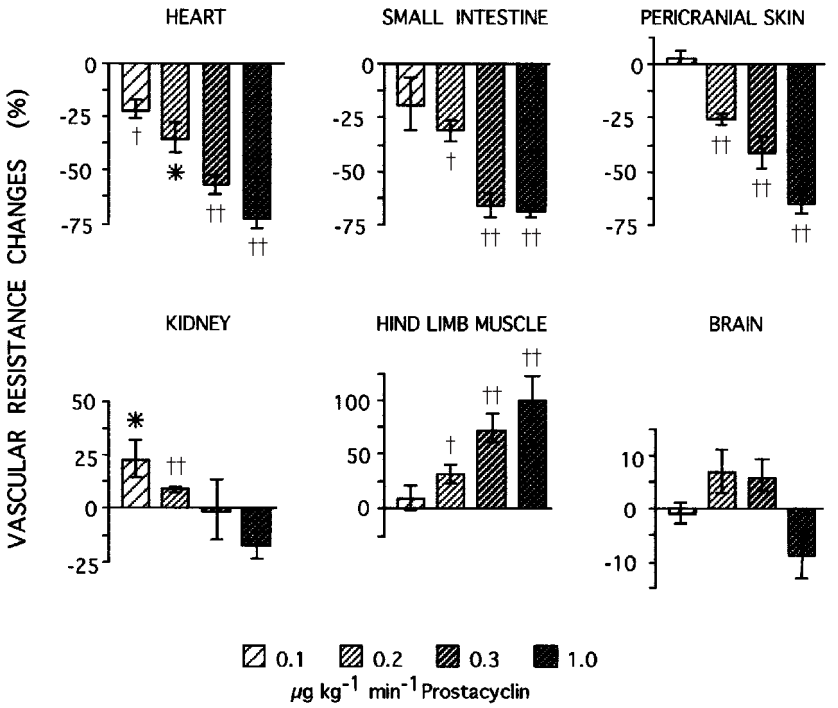

Figure 2 Regional vascular resistance changes (percentage regarding the corresponding control value) produced by the i.v. infusion of prostacyclin 0.1, 0.2, 0.3 and 1.0 μg kg^{-1} min^{-1}. The columns represent the geometric mean values and the bars the s.e.mean obtained after converting back the logarithmic transformed individual data. *$P<0.05$; †$P<0.01$ and ††$P<0.001$ with respect to zero change.

coronary blood flows decreased 20–30% (Muhvich *et al*., 1992). Consequently, the increase from 94 to 106 mmHg observed by us during 1.0 μg kg^{-1} min^{-1} i.v. PGI_2 is too small to alter significantly the blood flow to those tissues. Although cerebral vessels are very sensitive to hypercapnia and hypoxia (Faraci & Heistad, 1998), the changes observed in blood gases tension during the highest dose of PGI_2 are quite modest to induce a substantial modification of the cerebral blood flow that was reduced too by the lower PGI_2 doses, which did not change the blood gases tension.

Coronary flow in isolated perfused rat hearts was increased both by PGI_2 and iloprost (Fisher *et al*., 1987). We have found strong dose-dependent coronary vasodilation in response to i.v. PGI_2 that led to great increases of blood flow to the myocardium. This confirms in the rat, *in vivo*, the coronary vasodilation described *in vitro* as well as that reported in other animal species (Moncada & Vane, 1979) and reinforced the hypothesis for a physiological role of PGI_2 (Altman & Bache, 1994), which is the major prostaglandin produced in the rat heart (De Deckere *et al*., 1977). PGI_2 decreases heart work in anaesthetized cats (Lefer *et al*., 1978). Since we found dose-related decreases of MAP, that is, decreases of the afterload, we suggest that the increase of coronary blood flow produced by PGI_2 was not secondary to an increase in heart work but it could be, almost in part, the result of a direct dilation of coronary vessels which are highly sensitive to this prostaglandin. Although it has been recently proposed that PGI_2 has no direct coronary vasodilator effect (Kieler-Jensen *et al*., 1996), a study performed in conscious dogs shows that coronary vasodilation induced by PGI_2 is due to two components: a reflex parasympathetic cholinergic action mediated by NO and a direct PGI_2 action (Zhao *et al*., 1996). Although it has been recently shown that PGI_2 reduced plasma concentration of nitrate in anaesthetized rats (Nava *et al*., 1996), the reduction was observed during marked fall in arterial blood pressure (approximately −42%) and was also produced by adenosine, a well known potent coronary vasodilator. Hence, it is difficult to establish if the prostacyclin-induced coronary vasodilation observed in our experiments could be limited by a reduction in the plasma concentrations of nitric oxide. Finally, other actions of PGI_2, as the inhibition of the production of the potent vasoconstrictor endothelin in the cardiac tissue (King *et al*., 1996) or the release of adenosine (Blass *et al*., 1980), might also contribute to the observed effects on the coronary circulation.

Relaxation of mesenteric strips by PGI_2 was initially reported by Bunting *et al*. (1976). The i.v. infusion of iloprost produced dose-dependent increases of mesenteric blood flow in conscious rats (Steinberg *et al*., 1988). However, Agdeabo & Malik (1990) found in isolated rat mesenteric arteries preconstricted with norepinephrine or vasopressin that PGI_2 produced a biphasic effect, constriction then prolonged dilation. Both components of the response were dependent upon endothelium. Consequently, the strong dose-related decreases of vascular resistance and increases of blood flow observed by us in the small intestine are consistent with the effects obtained with iloprost in rats (Steinberg *et al*., 1988), and with the PGI_2-induced mesenteric dilatation observed in other experimental conditions and animal species (Bunting *et al*., 1976; Smith *et al*., 1978) and demonstrated that also in the awake rat the mesenteric arterial tree is highly sensitive to the PGI_2 dilating activity.

On the contrary, we found rat renal vessels not to dilate in response to PGI_2. Conflicting results have been reported concerning the renal haemodynamic effects of PGI_2 in various animal species. Thus, an increase of total renal blood flow was observed in anaesthetized dogs (Hill & Moncada, 1979), while no change was reported in anaesthetized cats (Smith *et al*.,

1978). Baer *et al.* (1979) found in anaesthetized rats that renal vascular resistance was unchanged during i.v. infusion of PGI_2 ($0.05-0.5$ $\mu g\ kg^{-1}\ min^{-1}$) and the decreases of renal blood flow they observed were the consequence of the fall in arterial pressure. A decrease of renal blood flow during PGI_2 infusion was also reported by Gerber & Nies (1979). However, Steinberg *et al.* (1988) reported that i.v. infusion of iloprost increased renal blood flow and decreased renal vascular resistance in awake rats. We found a renal vasoconstriction and decreased renal blood flow during the infusion of the low PGI_2 doses but no significant modifications during the infusion of the medium and high doses. Since PGI_2 releases renin from the renal cortex (Frölich & Walker, 1980) and angiotensin II increases renal vascular resistance and decreases renal blood flow in awake rats (Hsu *et al.*, 1980), it is possible that this humoral factor contributes to the net effect we observed during infusion of PGI_2. It is interesting to note that in rats, subvasodepressor doses of PGI_2 increased total renal arteriolar resistance but administration of saralasin, an angiotensin II antagonist, transformed the response of renal arterioles to PGI_2 in vasodilation (Schor & Brenner, 1981). We suggest that during the i.v. infusion of 0.1 and 0.2 $\mu g\ kg^{-1}\ min^{-1}$ PGI_2 to awake rats, the rise in the intrarenal levels of angiotensin II might lead to a net decrease of renal blood flow while the higher doses of PGI_2 should be able to counteract the angiotensin II vasoconstrictor effect.

We observed a dose-related constriction of skeletal muscle vessels (from the femoral territory) in response to PGI_2 infusion and corresponding decrease of blood flow. This is compatible with a lack of direct vasodilator effect of PGI_2 on these vessels while activation of neuro-humoral factors might be responsible for the constrictor effect observed. In addition to the commented release of renin, PGI_2 may activate sympathetic reflex in the conscious rat (Steinberg *et al.*, 1988), may induce the generation of cyclo-oxygenase derived vasoconstrictor substance by rat aortic tissue (Van Dam *et al.*, 1986) and decrease the plasma level of nitric oxide (Nava *et al.*, 1996). These factors might contribute to the vasoconstriction and reduction of hind limb muscle blood flow observed during the infusion of PGI_2. As shown in rabbits by Förstermann *et al.* (1984), PGI_2 is unable to modify the femoral activity to noradrenaline and probably does not modulate the contractility of femoral vessels. In addition, our results are in agreement with the lack of reactivity to i.v. PGI_2 observed in human muscular vessels (Wollersheim & Thien, 1988). Nevertheless, i.v. PGI_2 infusion increased skeletal muscle blood flow in pentobarbitone anaesthetized cats (Smith *et al.*, 1978). Differences in species and experimental conditions may explain this divergence. For instance, prostaglandin $F_{2\alpha}$, which had little effect on resistance vessels of skeletal muscle (Malik & McGiff, 1976) and potentiated the noradrenaline vasoconstriction in rabbit femoral vessels (Förstermann *et al.*, 1984), was also found to increase skeletal muscle blood flow in anaesthetized cats by Smith *et al.* (1978). Steinberg *et al.* (1988) reported that in awake rats i.v. iloprost increased hind limb flow even in subdepressor doses. However, they measured flow using a probe in lower aorta and the modest effects they found might be the consequence of a vasodilation in the skin of the hind limb.

The sensitivity of the pericranial skin vessels to the vasodilating effect of PGI_2 observed by us in the awake rat confirms the report of Weeks & Compton (1979) about flushing of the pinnae during prolonged i.v. infusions of this prostaglandin. This is similar to that described in human volunteers (Brown & Pickles, 1982; Wollersheim & Thien, 1988) or in patients with severe Raynaud's phenomenon (Kingma *et al.*, 1995), whose more frequent response to PGI_2 administration was facial flushing. This was also extremely common after the administration of iloprost (Grant & Goa, 1992). Moreover, in the rat, the i.v. administration of a stable PGI_2 analogue also dilate preferentially skin vessels (Sawada *et al.*, 1995). It is reasonable to assume that vasodilation of skin vessels may contribute positively to the improved survival of skin flaps observed in rats (Emerson & Sykes, 1981) and humans (Renaud *et al.*, 1996).

We have found that i.v. infusion of PGI_2 in a relatively wide range of doses that give biologically active concentrations in the peripheral circulation does not dilate cerebral vessels in the awake rat and, as MAP falls, cerebral blood flow is reduced. It is known that PGI_2 dilated pial arterioles *in vivo* and cerebral vessels *in vitro* from different animal species and humans (Pickard, 1981) but had no effect on rat pial vessels until concentrations of the micromolar range were reached, which induced constriction (Uski *et al.*, 1981). In addition, PGI_2 reduced cerebral blood flow after i.v. administration to normal humans (Brown and Pickles, 1982), did not change cerebral blood flow in septic patients (Berre *et al.*, 1994), and it modified neither the basal cerebral blood flow nor the blood flow and its regulation after global cerebral ischaemia in anaesthetized cats (Kerckhoff *et al.*, 1983). The intracarotid administration of iloprost, an analogue of PGI_2, increased blood flow to an intracerebral transplanted rat tumour without significant changes of the regional cerebral blood flow in the ipsilateral hemisphere and of the systemic arterial pressure (Nomura *et al.*, 1993). Then, it is not surprising that we found no dilating effect of PGI_2 on cerebral blood vessels in awake rats, although, the dose-related decrease of cerebral blood flow was unexpected.

Since the concentration of 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) was higher in cortical tissue of rats with haemorrhagic hypotension than in control normotensive rats, it was proposed that PGI_2 may play a role in autoregulation (Shohami & Sidi, 1984). However, in our experiments cerebral blood flow was decreased during PGI_2 infusions and there is a significant correlation between the degree of the reductions of cerebral blood flow and those of MAP. Hence, we suggest that PGI_2 might interfere with cerebral autoregulation in awake rats. This was also suggested in humans (Brown & Pickles, 1982). Since during infusion of the high dose of PGI_2 we observed a slight degree of hyperventilation, it cannot be completely discarded this factor had some influence. However, it seems that the small changes produced in blood gases tension were insufficient to account for the entire phenomenon and for the lack of autoregulation observed.

It has been repeatedly proposed that PGI_2 may play a role in the regulation of cerebral circulation under different conditions (Faraci & Heistad, 1998; Pickard, 1981; Quintana *et al.*, 1983). However, our results in awake rats and the above commented reports in other animal species and in humans, clearly show that direct evidence is needed prior to assigning a physiological or pathophysiological role to PGI_2 in the control and regulation of cerebral blood flow.

On the other hand, there is a regional variability in PGI_2 production with a greater activity in the more proximally or centrally located vessels (Fann *et al.*, 1989), prostaglandin E_2 production increases in relation to PGI_2 as vessel diameter decreases (Gerritsen, 1987) and PGI_2 must be considered a local hormone rather than a circulating one (Vane *et al.*, 1990). Since both quantitative and qualitative differences have been found by us in regional vascular reactivity to PGI_2, its true role in the regulation of regional blood circulation as well as the effectiveness of exogenous PGI_2 to enhance blood perfusion in

compromised areas depend on both the animal species and the vascular bed considered.

We conclude that, in awake rats, the coronary vessels are extremely sensitive to the vasodilating effect of PGI_2 and that the mesenteric vessels and those of the pericranial skin are very responsive too. In addition, cerebral resistance vessels do not dilate during i.v. infusion of PGI_2 and autoregulation is inefficient to maintain cerebral blood flow which decreases subsequently to the fall in MAP.

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