Biochemical Pharmacology, Vol. 28, pp. 3161 3166.
Pergamon Press Ltd. 1979. Printed in Great Britain.

COMMENTARY

PROSTACYCLIN AS A CIRCULATORY HORMONE

RYSZARD J. GRYGLEWSKI
Department of Pharmacology, Copernicus Medical Academy, 31-531 Cracow, 16 Grzegórzecka, Poland

Prostaglandins (PGs) are *chemically* stable products of cyclo-oxygenation of dihomo- γ-linolenic, arachidonic (AA) and all-Z-5,8,11,14,17-icosapentaenoic acids. Some PGs ($PGF_{2\alpha}$) raise while others (PGE_1, PGE_2) lower blood pressure, some (PGE_1, PGE_2, PGD_2) inhibit and others (PGE_2) enhance blood platelet aggregability. These vascular and platelet effects of PGs should be regarded as their *pharmacological* performance. Although there is a chance that PGE_1, PGE_2 and PGD_2 are generated in the blood or in vascular walls, the main products formed from AA in the cardiovascular system are thromboxane A_2 (TXA_2) [1] and prostacylin (PGI_2) [2, 3].

Microsomal preparations from a number of mammalian tissues convert AA to cyclic endoperoxides (PGG_2 and PHG_2). These unstable intermediates may give rise to PGs; however, platelet microsomes also contain a thromboxane synthetase which isomerizes PGH_2 to TXA_2 [4], and arterial microsomes are rich in a prostacyclin synthetase which converts PGH_2 to PGI_2 [2, 3]. TXA_2 and PGI_2 are unstable in biological fluids. At 37° and at pH 7.4, TXA_2 is broken down ($1/2t = 30$ sec) to thromboxane B_2 (TXB_2) and PGI_2 is decomposed ($1/2t = 3$–5 min) to 6-oxo-prostaglandin F_1 (6-oxo-$PGF_{1\alpha}$). TXA_2 is a powerful vasoconstrictor and promotes platelet aggregation, whereas PGI_2 dilates arteries and prevents platelets from aggregating. The stable products of the chemical decomposition of TXA_2 and PGI_2 are biologically inactive.

Apart from its cyclo-oxygenation, AA is a substrate for animal and plant lipoxygenases [5], giving rise to a number of hydroperoxy acids. AA and other polyunsaturated fatty acids are also easily auto-oxidized [5]. The resulting lipid peroxides inhibit prostacyclin synthetase ($IC_{50} = 2$–$4\ \mu M$) [3, 6]. We put forward a hypothesis that an increase in lipid peroxidation promotes intravascular thrombogenesis and atherosclerosis by decreasing the PGI_2:TXA_2 ratio in the circulatory system [3, 7]. Indeed, an atherogenic diet in rabbits causes a dramatic suppression of PGI_2 generation by the arteries and the heart [7], while human atherosclerosis is associated with an enhanced capacity of platelets to generate TXA_2 [8]. Furthermore, we have recently shown [9] that the clinical symptoms of advanced *arteriosclerosis obliterans* are rapidly alleviated by a long lasting infusion of PGI_2. The above findings indicate that PGI_2 is an antiatherosclerotic hormone.

If PGI_2 plays the suggested role, then it should be continuously generated in the body. Thereby, the following question has to be put forward. Is PGI_2 only a local hormone which is occasionally synthetized by vascular endothelium during its "bombardment" with blood platelets, or is PGI_2 also a circulating hormone which continuously keeps platelets in a "non-aggressive state" as well as regulating the capillary blood flow? I shall present evidence in favour of this second alternative.

PROPERTIES OF PGI_2

A powder of prostacyclin sodium salt [10] is stable when stored at 4°. The stability of PGI_2 in aqueous solution depends on pH and temperature. When dissolved in a buffer, pH 10.5, PGI_2 can be stored at 4° for several days without appreciable loss of its biological activity.

PGI_2 is a vasodilator which lowers arterial blood pressure in man and in animals, and is 4–8 times more potent than PGE_2, and 100 times more active than 6-oxo-$PGF_{1\alpha}$. In man, intravenous infusion of PGI_2 (2–20 ng/kg/min) causes erythema which covers the face, neck, palms and feet, accompanied by a rise in the skin temperature of these regions. PGI_2 at a dose of 50 ng/kg/min may result in a circulatory collapse. These circulatory effects of PGI_2 wane within a couple of minutes after termination of its infusion [11, 12]. PGI_2 is equipotent as a vasodilator when administered either intra-arterially or intravenously. This indicates lack of pulmonary removal of PGI_2, unlike PGE_1, PGE_2 or $PGF_{2\alpha}$, which are rapidly taken up and metabolized in the lungs, and are therefore much less active when administered intravenously [13].

PGI_2 is the most potent and the most universal endogenous inhibitor of platelet aggregation so far discovered [2, 3]. It is 20 times more potent than PGD_2 and 40 times more potent than PGE_1 in preventing platelet aggregation in human platelet rich plasma [14]. The anti-aggregatory properties of PGI_2 are closely associated with stimulation of platelet adenylate cyclase [15]. In hamsters and in rabbits PGI_2 prevents experimental thrombus formation *in vivo* [16]. In man, PGI_2 (5–10 ng/kg/min, i.v.) suppresses platelet aggregability and disperses the circulating platelet aggregates [11, 12]. This last effect—let us call it a deaggregatory action of PGI_2—has also been seen in animal experiments and it is potentiated by phosphodiesterase inhibitors [17].

ASSAY OF PGI_2

When decomposed spontaneously, PGI_2 can be assayed as 6-oxo-$PGF_{1\alpha}$ by radiochemical [18], radioimmunoassay [19] or mass spectrometric [20] techniques. *In vivo*, PGI_2 seems to be metabolized to several other products as well as 6-oxo-$PGF_{1\alpha}$ and therefore

the assay of 6-oxo-$PGF_{1\alpha}$ in blood and in urine may not reflect the total turnover of PGI_2 in the body.

Bioassay is less precise but more versatile than physicochemical techniques and it has an advantage of detecting *in statu nascendi* biologically active PGI_2. *In vivo* bioassay of PGI_2 is based on either its vasodilator or its deaggregatory properties. When superfused with blood or with Krebs' solution, a spirally cut strip of bovine coronary artery is relaxed by PGI_2 in a dose-dependent manner [21]. PGs and TXA_2 contract this vascular strip or have no effect on its tone. Alternatively, a strip of collagen tissue (e.g. Achilles' tendon of a rabbit) is superfused with heparinized blood and its weight is continuously monitored. The collagen strip gains in weight owing to the deposition of platelet clumps. This increase in weight reaches a plateau after 30–40 min of blood superfusion [22]. PGI_2 causes a dose-dependent loss in weight of the strip since PGI_2 deaggregates the superficial layers of platelet aggregates [23]. PGD_2, PGE_1 and adenosine have similar deaggregatory properties. These substances are less potent then PGI_2 by factors of 30, 60 and 10,000, respectively. Out of these deaggregatory agents, only PGI_2 is unstable in extracorporeal circulation. The instability of PGI_2 offers an easy way for an additional confirmation of its biological identity. A pair of blood superfused vascular or collagen strips are arranged in line with a delay coil (37°) in between them. The lower strip will show less biological activity of PGI_2 than the upper one. The identity of PGI_2 can also be confirmed by antiserum raised against 5,6-dihydro-PGI_2 [24]. When added to superfusing blood the anti-serum should abolish biological responses to PGI_2 [25].

A CONCEPT OF HORMONAL PGI_2

We put forward a hypothesis that the pulmonary endothelium may be considered as a huge "endocrine-like gland" which continuously secrets PGI_2 into arterial blood [23, 26]. Unstable PGI_2 is efficiently removed from peripheral but not in pulmonary circulation [13] and thus there arises a difference in concentration of PGI_2 between arterial and venous blood. Coronary and cerebral arteries are at the closest vicinity to the source of hormonal PGI_2 and they will benefit most of all from the endocrine function of the lung. It is supposed that "circulating PGI_2" supports the anti-aggregatory action of "vascular PGI_2" which is locally generated by arterial endothelium. Hormonal PGI_2 may also counteract vasoconstrictor effects of circulating hormones such as angiotensin, vasopressin or catecholamines. The protective function of hormonal PGI_2 will become of the ultimate importance when the generation of PGI_2 by arteries is suppressed (e.g. atherosclerosis) or when the lungs are stimulated to increase their production of PGI_2 (e.g. hyperventilation, pulmonary embolism, rise in plasma AA level, activation of the renin–angiotensin system).

The regulatory mechanisms which control the secretion of PGI_2 are presently under investigation. Recently, a possibility has emerged that not only the lungs but also the kidneys and brain may release PGI_2 into the circulation. A hormonal link between kidneys and lungs is of special interest. Both organs generate PGI_2 in response to stimulation with angiotensin II. On the other hand, renal release of renin is triggered by PGI_2 [27]. We are far from understanding this peculiar relationship; however, we have experimental evidence for the dependence of the amount of circulating PGI_2 on the degree of activation of the renin–angiotensin system.

PERFUSED LUNGS RELEASE PGI_2

The lungs have long been recognised as the organs which rapidly transform AA to more polar products. Presently, there is no single metabolite of AA which would not be proposed as a major product generated by lungs. Indeed, TXA_2, PGs, products of lipoxygenation of AA and their metabolites are generated by the lungs in response to anaphylactic shock, during intoxication with high doses of histamine, bradykinin, 5-hydroxytryptamine and AA, as well as when lung tissue is squeezed, scratched, vibrated, chopped, minced or homogenized. They are not generated when the lung is gently perfused or in a normal state.

We have shown [23, 26] that mildly inflated isolated lungs of cats, rabbits, guinea pigs and rats spontaneously release a PGI_2-like material when perfused through pulmonary artery with Krebs' solution (37°, 2–6 ml/min). TXA_2 and PGs are not detected in the effluent. PGI_2-like material in the lung perfusate is detected as an unstable substance that relaxes a strip of bovine coronary artery [13, 21] and deaggregates platelet clumps [17, 24] (see Assay of PGI_2). The generation of this substance in perfused lungs is blocked by cyclo-oxygenase inhibitors, and is stimulated by low concentrations of AA (100 ng/ml) and angiotensin II (10–100 ng/ml). Using a combination of bioassay and mass spectrometric techniques, we have shown that PGI_2 like activity from the cat lung perfusate has the same chemical ionisation spectrum as that of authentic 6-oxo-$PGF_{1\alpha}$. Quantification of 6-oxo-$PGF_{1\alpha}$ by multiple ion detection has revealed that perfused cat lungs spontaneously release PGI_2 at a concentration of 2–4 ng/ml and this concentration is doubled during an infusion of angiotensin II into the pulmonary artery (Fig. 1).

Most other biogenic peptides and amines at concentrations up to 300 ng/ml do not release PGI_2 from perfused lungs. Inactive compounds include vasopressin, oxytocin, neurotensin, ceruloplasmin, pentagastrin, secretin, VIP, eledoisin, enkephalin, somatostatin, glucagon, calcitonin, adrenaline, noradrenaline, 5-hydroxytryptamine and histamine. Only bradykinin (1–5 ng/ml) is a potent releaser of PGI_2 from perfused guinea-pig lungs. Angiotensin I is half as potent as angiotensin II. An inhibitor of converting enzyme—SQ 14 225 (100 ng/ml) [28]—prevents the PGI_2-releasing action of angiotensin I and has no effect on the activity of angiotensin II. The latter is blocked by 1-sarcosine,8-alanine angiotensin II (saralasin 20 ng/ml), which is a receptor antagonist of angiotensin II [29].

In summary, the isolated perfused lungs of four animal species continuously produce PGI_2. PGI_2 is the only product of cyclo-oxygenation of AA that is spontaneously released into the lung perfusate. TXA_2 and PGs are generated by the lungs in response to pathological stimuli, including high concentrations of AA. A selective enhancement of PGI_2 released from perfused

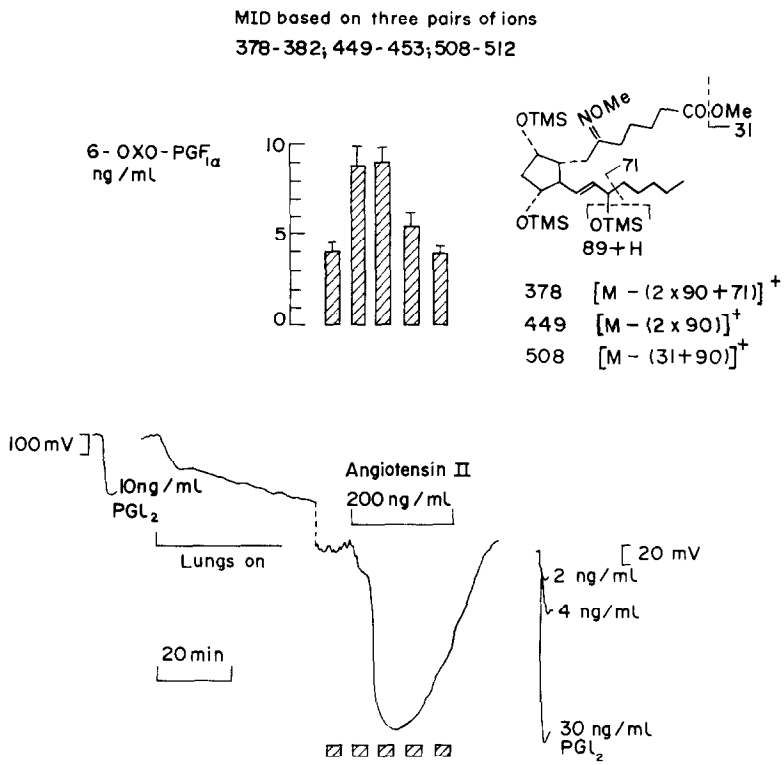

Fig. 1. Spontaneous and angiotensin II-induced release of PGI_2 from isolated, perfused (3 ml/min) cat lungs. The effluent from lungs superfused a strip of bovine coronary artery (lower tracing), which was treated with indomethacin (1 μg/ml) and calibrated with synthetic PGI_2 (2–30 ng/ml). A change from superfusing Krebs' solution for the lung perfusate is denoted as "lungs on". The squares at bottom of the tracing mark collection times of the effluent for mass fragmentography. To each sample 1 μg of 3,3,4,4-D_4-6-oxo-$PGF_{1\alpha}$ was added as a carrier. The samples were extracted, purified by TLC and derivatized. The quantities of 6-oxo-$PGF_{1\alpha}$ in each sample (columns) were calculated automatically by multiple ion detection (MID) software system as based on 3 pairs of ions.

lungs is achieved by infusions into the pulmonary artery of low concentrations of AA, angiotensin and bradykinin. These findings prompted us to check whether PGI_2 is also released from lungs *in vivo*.

IS PGI_2 A CIRCULATING HORMONE?

In order to prove that PGI_2 is secreted by the lungs into the circulation, one has to detect a difference of PGI_2 concentration between arterial and venous blood. Using the bloodbathed collagen strip technique [22] (see Assay of PGI_2), we have found that in anaesthetized cats an intravenous injection of synthetic PGI_2 results in a more pronounced deaggregation in arterial blood than in venous blood [23, 26]. Since we excluded other possibilities [23] our final conclusion was that the biological activity of exogenous PGI_2 is enhanced after its passage through the pulmonary circulation, because of the additive effect with endogenous PGI_2 which is generated by the lungs and then destroyed in peripheral circulation. Moncada *et al.* [25] observed a similar phenomenon in rabbits. When using an antiserum raised against 5,6-dihydro-PGI_2 which cross-reacted with PGI_2, Moncada *et al.* [25] have clearly shown that the deposition of platelet clumps on blood superfused collagen strips is restricted by the presence in the blood of an anti-aggregatory substance, the action of which is neutralized by the antiserum [24]. Furthermore, the concentration of this PGI_2-immunoreactive substance in arterial blood is higher than that in venous blood.

There are other data which should be mentioned. The lungs are stimulated to release additional amounts of a PGI_2-like substance into arterial blood during hyperventilation or during pulmonary embolism. In both cases, a PGI_2-like substance can also be assayed as a relaxation of a strip of bovine coronary artery (Fig. 2).

To summarize, using bioassay and immunoassay techniques it has been shown that in cats and rabbits there circulates a PGI_2-like substance, the concentration of which is higher in arterial blood than that in venous blood. The release of PGI_2 from lungs is stimulated by hyperventilation and by pulmonary embolism. The data concerning the spontaneous release of PGI_2 by lungs *in vivo* might be open to criticism because we used blood-superfused collagen strips as the detectors of PGI_2. It is possible that aggregating platelets were removed from collagen strips and brought with the blood stream to the pulmonary circulation. If so, the resulting pulmonary platelet emboli might stimulate the lungs to generate PGI_2. The purposely stimulated release of PGI_2 from lungs to arterial blood escapes this criticism since its occurence has also been demonstrated by the relaxation of a strip of bovine coronary artery (Fig. 2). The most convincing evidence for hormonal function of lungs *in vivo* would be the demonstration of an arterial–venous difference in the concen-

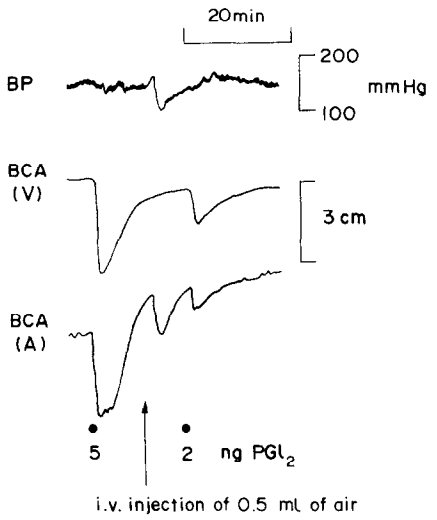

Fig. 2. The release of PGI_2 into arterial circulation by pulmonary embolism. In anaesthetized cat (40 mg/kg sodium nembutal) aortic (A) and mixed venous (V) blood superfused (2 ml/min) two strips of bovine coronary artery (BCA) and were returned into the animal. Arterial blood pressure (BP) was registered. Both arterial strips were relaxed to a similar degree by synthetic PGI_2 (2 ng and 5 ng) injected into the superfusing blood. Pulmonary embolism was induced by an intravenous injection of 0.5 ml of air. PGI_2-like activity appeared in arterial but not in venous blood.

tration of 6-oxo-PGF_1 by mass spectrometry. This is a somewhat complicated problem because the biotransformation of PGI_2 is not fully understood. Meanwhile, we decided to stimulate the release of PGI_2 in anaesthetized cats by infusions of angiotensin II—a peptide which has been found to be a powerful stimulator of PGI_2 generation in perfused cat lungs (Fig. 1).

ANGIOTENSIN RELEASES PGI_2 INTO CIRCULATION

Following intravenous injection of angiotensin I or angiotensin II at doses of 100–300 ng/kg into anaesthetized cats, there appears in the blood a substance which relaxes strips of bovine coronary artery. The peak relaxation of coronary arteries can be matched with relaxation induced by PGI_2 (5–10 ng/ml) infused over assay organs. The released substance is unstable, has deaggregatory action and its generation is suppressed by cyclo-oxygenase inhibitors. These findings indicate that angiotensin I and angiotensin II stimulate the release of PGI_2 into the circulation.

There is an observation which does not fit to a concept that *in vivo* angiotensin releases PGI_2 exclusively from lungs. After intravenous infusion of angiotensin II we could not detect a difference in concentration of PGI_2-like substance in arterial vs venous blood. Indeed, further experiments have shown that angiotensin II releases a PGI_2-like substance not only when infused intravenously but also when infused into the renal artery and into the carotid artery (Fig. 3). Therefore, *in vivo* angiotensin seems to be a releaser of PGI_2 from at least three organs—lungs, kidneys and brain—or from the blood-vessels which supply these organs. Interestingly enough kidney and brain contain renin–angiotensin system [30], while the lungs are rich in an enzyme that converts angiotensin I to angiotensin II. Isolated perfused mesenteric blood vessels of rabbits also release PGI_2 when constricted by angiotensin II. Could it be that a "hypertensive" renin–angiotensin system activates "hypotensive and anti-aggregatory"

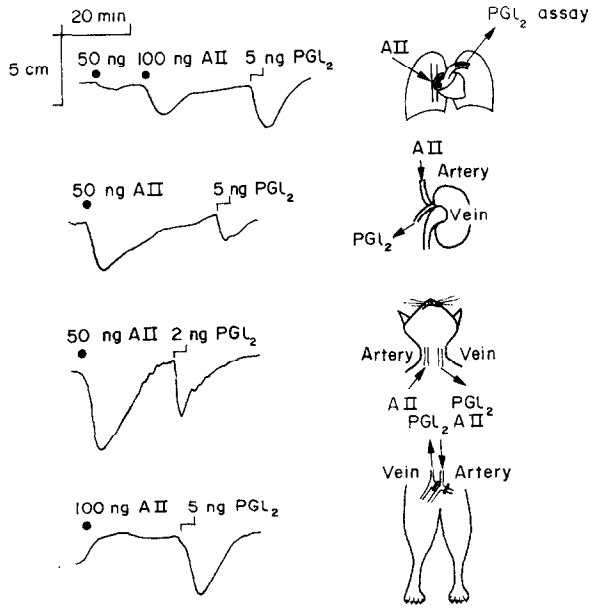

Fig. 3. The release of PGI_2-like activity by injection of angiotensin II (A III) into various vascular beds of anaesthetized cats as detected by relaxation of blood-superfused strips of bovine coronary artery. The sites of angiotensin II infusion and the sites of PGI_2 detection were as follows. Right atrium and ascending aorta; renal artery and renal vein; right carotid artery and left jugular vein; right femoral artery and right femoral vein. The sensitivity of the assay organs was checked by infusions of synthetic PGI_2 into the superfusing blood.

prostacyclin system? Does this activation occur only in case of emergency or is it a physiological regulatory mechanism? The first possibility is doubtless. Below we present indirect evidence that blood level of PGI_2 depends on the degree of activation of renin–angiotensin systems.

Inactivation of converting enzyme by SQ 14 225 (0.5 mg/kg) inhibits the release of PGI_2 by angiotensin I, whereas saralasine (0.2 μg/kg) [29] blocks the releasing properties of both angiotensins. These findings clearly demonstrate that from the renin–angiotensin system only angiotensin II is responsible for the release of PGI_2 into the circulation. This statement prompted us to use SQ 14 225 and saralasin as the pharmacological tools in order to interfere with the endogenous renin–angiotensin system and to observe whether this interference will influence blood levels of PGI_2. We used anaesthetized cats with extracorporeal circulation and blood superfused collagen strips [22]. After the deposited platelet aggregates reached a plateau, SQ 14 225 or saralasin were injected intravenously. In both instances a further distinct increase in deposition of platelet clumps occured. A similar phenomenon was observed by Moncada *et al.* [25] after removal of PGI_2 from blood by its neutralization with a specific antiserum. We believe that inhibition of angiotensin II biosynthesis (SQ 14 225) or invalidation of its receptor interactions (saralasin) leads to a decrease in PGI_2 release into circulation. Therefore, it is conceivable that the degree of activation of the renin–angiotensin system is one of the factors responsible for blood levels of PGI_2.

In summary, *in vivo* angiotensin II releases PGI_2 into the circulation, probably from vascular beds of the lungs, kidneys and brain. It may well be that endogenous angiotensin II is a physiological stimulator of PGI_2 secretion. On the other hand it has been demonstrated that PGI_2 stimulates renin release from kidney [27]. Presently, it is difficult to define the significance of this positive feedback loop between the renin–angiotensin and prostacyclin systems. Possibly, we lack knowledge of an intermediate link. Bartter's syndrome [31] might represent a specific case of distortion of this regulatory mechanism.

CLINICAL EVIDENCE

There is no direct evidence that PGI_2 is a circulating hormone in man; however experimental data strongly suggest this possibility, as well as an idea that atherosclerosis is a disease caused by deficiency of PGI_2 [7]. On the basis of the above assumptions we decided to simulate endogenous secretion of PGI_2 by a long lasting infusion of synthetic PGI_2 into patients suffering from advanced *arteriosclerosis obliterans* of the lower extremities [9]. A dose of 5 or 10 ng/kg/min of PGI_2 was infused into the femoral artery over a period of 72 hr. In five patients, diagnosis of *arteriosclerosis obliterans* was confirmed by angiography, resting pain, ischemic ulcers and focal necorsis of toes and heel regions which persisted from 3 months to 3 years, before PGI_2 therapy. Conservative treatment had been unsuccessfully tried in the past and no further help other than amputation could be offered. Within 2 days after termination of the PGI_2 infusion, pain disappeared in all patients and did not return during the next 5 months of the observation period. In three out of five patients, complete regression of necrosis and healing of ischemic ulcers occured within 4–8 weeks after termination of the PGI_2 infusion. In the remaining two patients a considerable improvement was observed. Muscular blood flow as measured by ^{133}Xe clearance was increased by 170–430 per cent both during PGI_2 infusion and for the 6 weeks of measurement after its termination.

The striking clinical improvement following PGI_2 infusion can be considered as the result of the substitution therapy—we supplied the atherosclerotic patients with a hormone, the generation of which had been suppressed in their tissues for many months, and eventually this suppression resulted in the disease. Amounts of the infused PGI_2 were at "pharmacological" rather than at "physiological" range. Thereby, even a 72 hr period of infusion was sufficient for clearing obstructing platelet deposits from the capillaries or for stimulation of proliferation of new capillaries in the ischemic areas. Our clinical evidence is strengthened by the fact that until now, the vasodilator pharmacological treatment of advanced *arteriosclerosis obliterans* was totally unsuccessful.

SUMMARY AND CONCLUSION

PGI_2 is spontaneously released from isolated perfused lungs of cats, rabbits, guinea pigs and rats. This release is stimulated by arachidonic acid and by angiotensin II. In anaesthetized cats and rabbits there seems to exist a difference in PGI_2 concentration between arterial and venous blood. This difference can be increased by hyperventilation of lungs or by pulmonary embolism. Angiotensin II stimulates the release of PGI_2 into circulation; the lungs and also the kidneys and brain are the target organs for action of angiotensin. The activation of the renin–angiotensin system may be one of the mechanism which control the secretion of PGI_2 into the circulation. Whatever these mechanisms are, we believe that their impairment leads to the development of atherosclerosis, a disease believed to be caused by PGI_2 deficiency. Indeed, we have recently reported a rapid alleviation of the signs and symptoms of *arteriosclerosis obliterans* after prostacyclin therapy. The hypothesis that prostacyclin is a circulating hormone has gained further evidence; however, more experimental and clinical data are required to prove or disprove this possibility.

REFERENCES

1. M. Hamberg, J. Svensson and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994 (1975).
2. S. Moncada, R. J. Gryglewski, S. Bunting and J. R. Vane, *Nature Lond.* **263**, 663 (1976).
3. R. J. Gryglewski, S. Bunting, S. Moncada, R. J. Flower and J. R. Vane, *Prostaglandins* **12**, 685 (1976).
4. P. Needleman, S. Moncada, S. Bunting, J. R. Vane, M. Hamberg and B. Samuelsson, *Nature Lond.* **261**, 550 (1976).
5. P. W. Ramwell, E. M. K. Leovey and A. L. Sintetos, *Biol. Reprod.* **16**, 70 (1977).
6. J. A. Salmon, D. R. Smith, R. J. Flower, S. Moncada and J. R. Vane, *Biochim. biophys. Acta* **523**, 250 (1978).
7. R. J. Gryglewski, A. Dembińska-Kieć, A. Zmuda and T. Gryglewska, *Atherosclerosis* **31**, 385 (1978).
8. A. Szczeklik and R. J. Gryglewski, in *International Conference on Atherosclerosis* (Eds. L. A. Carlson, R.

Paoletti, C. R. Sirtori and G. Weber), pp. 597–605. Raven Press, New York (1978).
9. A. Szczeklik, S. Skawiński, R. Niżankowski, J. Szczeklik, P. Gluszko and R. J. Gryglewski, *Lancet* **1**, 1111 (1979).
10. N. Whittaker, *Tetrahedron Lett.* **32**, 2805 (1977).
11. A. Szczeklik, R. J. Gryglewski, R. Nizankowski, J. Musiaç, R. Piętoń and J. Mruk, *Pharmac. Res. Commun.* **10**, 545 (1978).
12. R. J. Gryglewski, A. Szczeklik and R. Nizankowski, *Thromb. Res.* **13**, 153 (1978).
13. G. J. Dusting, S. Moncada and J. R. Vane, *Br. J. Pharmac.* **64**, 315 (1978).
14. B. J. R. Whittle, S. Moncada and J. R. Vane, *Prostaglandins* **16**, 373 (1978).
15. P. R. Gorman, S. Bunting and O. V. Miller, *Prostaglandins* **13**, 377 (1977).
16. S. Moncada and J. R. Vane, in *Platelets: A Multidisciplinary Approach* (Eds. G. de Gaetano and S. Garattini), pp. 239–258. Raven Press, New York (1978).
17. R. J. Gryglewski, R. Korbut and A. Ocetkiewicz, *Prostaglandins* **15**, 637 (1978).
18. F. Cottee, R. J. Flower, S. Moncada, J. A. Salmon and J. R. Vane, *Prostaglandins* **14**, 413 (1977).
19. J. A. Salmon, *Prostaglandins* **15**, 383 (1978).
20. C. Pace-Asciak, *J. Am. chem. Soc.* **98**, 2348 (1976).
21. G. J. Dusting, S. Moncada and J. R. Vane, *Prostaglandins* **13**, 3 (1977).
22. R. J. Gryglewski, R. Korbut, A. Ocetkiewicz and T. Stachura, *Naunyn-Schmiedeberg's Arch. exp. Path Pharmac.* **302**, 25 (1978).
23. R. J. Gryglewski, R. Korbut, A. Ocetkiewicz, J. Spławiński, B. Wojtaszek and J. Święs, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmac.* **304**, 45 (1978).
24. S. Bunting, S. Moncada, P. Reed, J. A. Salmon and J. R. Vane, *Prostaglandins* **15**, 565 (1978).
25. S. Moncada, R. Korbut, S. Bunting and J. R. Vane, *Nature Lond.* **273**, 768 (1978).
26. R. J. Gryglewski, R. Korbut and A. Ocetkiewicz, *Nature Lond.* **273**, 765 (1978).
27. A. R. Whorton, K. Misono, J. Hollifield, J. C. Frohlich, T. Inagami and J. A. Oates, *Prostaglandins* **14**, 1095 (1978).
28. D. N. Harris, C. L. Heran, H. J. Goldenberg, J. P. High, R. J. Laffan, B. Rubin, M. J. Antonaccio and M. E. Goldberg, *Eur. J. Pharmac.* **51**, 345 (1978).
29. T. Hata, T. Ogihara, H. Mikami, H. Nakamaru, A. Maruyama, T. Mandai and Y. Kumahara, *Life Sci.* **22**, 1955 (1978).
30. D. Ganten and G. Speck, *Biochem. Pharmac.* **27**, 2379 (1978).
31. J. R. Gill, J. G. Fröhlich, R. E. Bowden, A. A. Taylor, H. R. Keiser, H. W. Seyberth, J. A. Oates and F. B. Bartter, *Am. J. Med.* **61**, 43 (1976).