Br. J. Pharmac. (1983), **79**, 019–021

Blood pressure changes in spontaneously hypertensive rats correlate with aortic prostacyclin formation

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1 The relationship between the blood pressure fall, induced by antihypertensive drugs or bleeding, and the formation of prostacyclin (PGI_2)-like activity in the thoracic aorta of spontaneously hypertensive rats has been investigated. Inhibition of ADP-induced platelet aggregation was used to assess PGI_2-like activity.

2 The decreases in blood pressure produced by clonidine, dihydralazine and prazosin were associated with increases of PGI_2-like activity of 50–80%. The increase in PGI_2-like activity correlated well with the blood pressure decrease, independently of the mechanism of the fall in blood pressure.

Introduction

Prostacyclin (PGI_2) shows a stronger hypotensive action than prostaglandin E_2 (PGE_2), especially in the spontaneously hypertensive rat (SHR) (Pace-Asciak, Carrara & Nicolaou, 1978). PGI_2 is the main prostaglandin released by the isolated aorta and its formation is higher in the aorta of SHR compared to normotensive rats (Pace-Asciak, Carrara, Rangaraj & Nicolaou, 1978).

In view of these results it seemed of interest to investigate the influence of antihypertensive drugs with different modes and sites of action on PGI_2 formation in the aorta of SHR under *ex vivo* conditions. In addition to these drug effects we have assessed the influence of blood pressure (BP) increase or decrease induced by changes in circulating blood volume on the *ex vivo* production of PGI_2-like activity by the aorta of SHR.

Methods

In expt. 1 male SHR (Okamoto strain, weighing about 300 g) were anaesthetized with pentobarbitone sodium (30 mg/kg, i.p.). Mean arterial BP was measured after cannulating the carotid artery via the pressure transducer EMT 34 and recorded on the Mingograf 81. The following substances (dissolved in twice-distilled water) were given by a single bolus injection in a volume of 1 ml/kg: dihydralazine sulphate 4.0 mg/kg, clonidine hydrochloride 0.05 mg/kg, prazosin hydrochloride 0.8 mg/kg and vehicle solution (controls). Twenty minutes after injection the animals were killed by decapitation and the thoracic aorta (descending branch) was removed quickly, cleaned and immediately deep frozen. The temperature and the time between freezing and thawing was held constant in order to standardize the substantial increase of PGI_2-like activity during this procedure.

A small piece of the cleaned aorta weighing about 10–15 mg was cut into three rings and rinsed quickly in ice cold Tyrode solution. The aortic rings were incubated in Tyrode solution (10 ml, 37°C, oxygenated, pH 8.4) for 20 min (maximum value of PGI_2-like production; half life time of PGI_2 in Tyrode solution 39 min) with shaking and without addition of substrate or cofactors.

The incubates (cooled to 4°C) were extracted rapidly with diethyl-ether at pH 5.6 (10 s shaking time). This pH was chosen for the extraction fluid because of the acid instability of PGI_2. A recovery rate for PGI_2 of 50% was mainly due to the poor partition of the compound into the ether phase (estimated by measuring the partition of PGE_1 under the same conditions).

The residue was redissolved in carbonate-NaCl solution (pH 8.6). The PGI_2-like activity was estimated in terms of inhibitory action on ADP-induced platelet aggregation. Platelet rich plasma (PRP) was prepared from citrated blood (3.14 w/v%, 1 part and 9 parts blood) of human volunteers. Platelet aggregation was measured at 593 nm by continuously recording the stirred buffer (carbonate-NaCl)-diluted PRP after 2 min of incubation (37°C) using a Specol

photometer with temperature controlled absorption equipment. The antiaggregatory potency of the samples was compared with that of authentic PGI_2 prepared from a stock solution immediately before use. The substance with antiaggregatory potencies contained in the samples had properties corresponding with PGI_2: it was inactivated after boiling for a short time and it lost its activity after acidification.

To investigate the influence of changes in the intravascular blood volume (expt. 2), SHR were prepared as described above. A volume of blood (3 or 5 ml) was withdrawn via a catheter introduced into the carotid artery. Another group of animals was given 5 or 7 ml of isotonic saline. Ten minutes later the animals were killed and prepared as described in expt. 1.

Statistical evaluation

Student's two tailed t test for paired samples was used for BP changes. Differences in PGI_2-like substance formation were estimated by two way variance analysis. Linear correlation was applied to changes in BP and PGI_2-like substance formation. The criterion for statistical significance was $P<0.05$. Results are expressed as means ± s.e.mean.

Drugs

The following were used: clonidine hydrochloride, dihydralazine sulphate, prazosin hydrochloride, PGI_2 (Wellcome Research Laboratory).

Results

The mean arterial BP of all SHR used in expt. 1 was 219.3 ± 3.9 mmHg. Twenty min after administration of antihypertensive drugs the fall in BP with dihydralazine was 103.8 ± 8.9 mmHg ($n=12$, $P<0.001$), with clonidine 65.7 ± 8.2 mmHg ($n=13$, $P<0.001$) and with prazosin 81.8 ± 8.7 mmHg ($n=12$, $P<0.001$). The BP of the control group was not significantly changed (−9.2 ± 5.5 mmHg).

The aortic PGI_2-like formation, measured *ex vivo*, in the control group was 55.2 ± 4.8 ng/mg wet weight ($n=10$). After administration of dihydralazine there was an increase of the aortic PGI_2-like formation of 79% ($n=7$, $P<0.05$), after clonidine of 70% ($n=9$, $P<0.05$) and after prazosin of 50% ($n=8$, $P<0.05$). There was a significant correlation between the changes in BP and the PGI_2-like formation, measured in the aorta, in the control group as well as in the groups after administration of antihypertensive drugs: for the control group $r=-0.76$ ($n=9$, $P<0.05$), for dihydralazine $r=-0.77$ ($n=8$, $P<0.05$), for clonidine $r=-0.73$ ($n=10$, $P<0.05$), for prazosin $r=-0.84$ ($n=8$, $P<0.01$) and for all SHR including the control group, $r=-0.75$ ($n=35$, $P<0.001$) – see Figure 1.

In expt. 2 the mean arterial BP for all SHR was 201.9 ± 7.4 mmHg ($n=14$). A blood loss of 3 or 5 ml caused a fall in BP of 102.9 ± 14.0 mmHg ($n=8$, $P<0.001$) and a PGI_2-like formation of 69.2 ± 9.7 ng/mg wet weight was measured in the aorta. The injection of isotonic saline seemed to cause no significant changes in BP (+9.0 ± 6.7 mmHg, $n=6$); the PGI_2-like formation in the aorta was 39.3 ± 3.3 ng/mg wet weight. Here too all changes in BP were significantly correlated to the respective values of PGI_2-like substance formed in the aorta ($r=-0.83$, $n=14$, $P<0.001$).

Discussion

A fall in BP induced by antihypertensive agents in SHR was associated with an increase in the formation of PGI_2-like substance in the aorta. This increase occurred independently of the accepted mode and site of action of the drugs and correlated well with the decrease in BP. Even a fall in BP, due to a reduction in the amount of circulating blood, was significantly correlated with an increased PGI_2-like substance formation. Moreover lesser, non-significant changes in BP in untreated SHR, correlated with the aortic PGI_2-like formation (Figure 1). These results indicate the independence of increased PGI_2-like substance formation from the type of mechanism decreasing BP.

An explanation for the interesting correlation is difficult at present and must initially be of a purely speculative nature. It is known that SHR display increased activity in the sympathetic nervous system (Judy, Watanabe, Henry, Besch, Murphy & Hockel, 1976) and that PGI_2 normalizes BP values increased by noradrenaline in rats (Okuno, Kondo, Suzuki & Saruta, 1980; Fischetti, Carmignani, Marchetti, Raneletti & Caprino, 1980). Possibly there is a negative feed back mechanism between PGI_2-like formation and the sympathetic activity as described by Schrör, Addicks, Darius, Ohlendorf & Rösen (1981). Because of the uncertainty about the significance of the circulating PGI_2 in the regulation of BP (Pace-Asciak, Carrara, Levine & Nicolaou, 1980) the interpretation of the results given appears feasible only when proposing a mutual effect between the PGI_2 formation in the aorta of SHR and the sympathetic activity at a local level.

We are grateful to Professor J.R. Vane (Wellcome Research Laboratory) for his generous supply of PGI_2 and to Dr H.-J. Hess (Pfizer, Central Research) for prazosin. It is a pleasure to acknowledge the competent technical assistance of Mrs H. Schmidt, Mrs S. Thümmel and Mrs E. Liedtke.

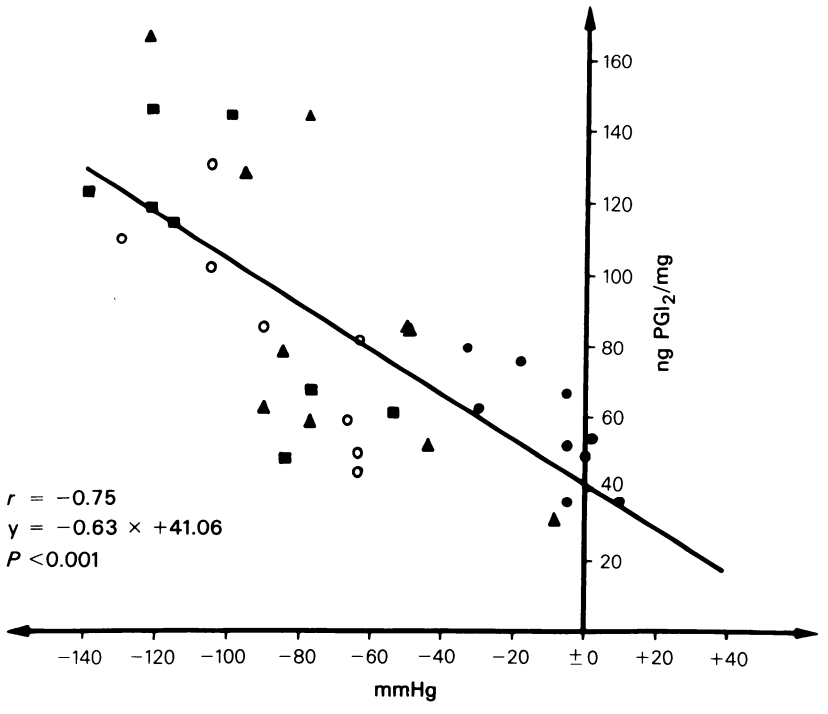

Figure 1 Correlation of blood pressure changes after administration of clonidine, dihydralazine, prazosin and vehicle solution (control) and the respective aortic formation of PGI_2-like substance in spontaneously hypertensive rats (SHR). Each point represents the blood pressure change 20 min after injection (abscissa scale) and PGI_2-like formation measured *ex vivo* (ordinate scale) of one animal: (●) control SHR; (▲) clonidine; (■) dihydralazine; (○) prazosin.

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(*Received November 19, 1982.*
Revised December 8, 1982.)