Br. J. Pharmac. (1980), **68**, 519–524

ATTENUATED DEPRESSOR RESPONSE TO ARACHIDONIC ACID AND PROSTAGLANDINS IN UNCLIPPED RENAL HYPERTENSIVE RATS

R.G.M. TEN BERG, W. DE JONG & F.P. NIJKAMP

Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, Utrecht, The Netherlands

1 In renal hypertensive rats (one-clip, two-kidney type) blood pressure returned to a normotensive level within 24 h after the removal of the renal artery clip (unclipping).

2 The decrease in blood pressure caused by intravenous administration of arachidonic acid, prostaglandin E_2 and prostaglandin I_2 was significantly reduced in these unclipped rats.

3 The hypotensive response to bradykinin and acetylcholine was the same in unclipped rats and in control rats.

4 Nephrectomy abolished the difference in blood pressure response to arachidonic acid and the two prostaglandins between unclipped and control rats.

5 An enhanced secretion of renin, as indicated by the absence of an increase in plasma renin activity, did not seem to be involved in the attenuated blood pressure response to arachidonic acid in unclipped rats.

Introduction

Prostaglandins may play a role in the regulation of blood pressure in normotensive and hypertensive animals (Vane & McGiff, 1975; Kaley, 1976; Dunn & Hood, 1977). Particular attention has been devoted to the role of prostaglandins in the pathogenesis of renal hypertension. The increase of blood pressure in renal hypertensive rats (Pugsley, Beilin & Peto, 1975; Schölkens & Steinbach, 1975; Nijkamp & De Jong, 1978) and in renal hypertensive rabbits (Romero & Strong, 1977) was reported to have been potentiated following blockade of prostaglandin synthesis. Within minutes after unilateral constriction of the renal artery of the dog, prostaglandins are released by the contralateral kidney in amounts sufficient to influence the vascular resistance in the muscles (Pamnani, Simon & Overbeck, 1976). A decrease in prostaglandin synthesis and concentration in the clipped kidney (Leary, Ledingham & Vane, 1974; Sirois & Gagnon, 1974; Smith & Somova, 1976) or in both kidneys (Pugsley, *et al.*, 1975) has been reported during the development of renal hypertension in rats. In one study with rats, however, an increase in renal prostaglandin synthesis of the clipped kidney was observed during the first 3 weeks after the application of the clip (Haux, Ingerowski & Von Bruchhausen, 1976). When the renal artery clip was removed from one-clip two-kidney hypertensive rats the blood pressure returned to a normotensive level within 24 h (Skulan, Brousseau & Leonard, 1974; Ten Berg, Leenen & De Jong, 1979). The reactivity of the blood pressure of these unclipped rats to reapplication of the clip and to renin, angiotensin or noradrenaline was enhanced at this time (Skulan, *et al.*, 1974; Ten Berg & De Jong, 1977). Prostaglandins may have an important part in the determination of vascular reactivity (for references see McGiff, Malik & Terragno, 1976). It appears that vascular responsiveness to a variety of vasocontrictor agents can be reduced by the release of prostaglandins (Messina, Weiner & Kaley, 1976), an event that may be a consequence of vasoconstriction, or may accompany it. On the other hand, several vasoconstrictor responses are potentiated after inhibition of endogenous prostaglandin synthesis (Messina, *et al.*, 1976). In the present study we investigated the responsiveness of unclipped rats to arachidonic acid (the endogenous precursor of the prostaglandins) prostaglandin E_2 and I_2, acetylcholine and bradykinin.

Methods

Experimental design

Male rats of a Wistar strain (Outbred stock, WU/Cpb-TNO, Zeist, the Netherlands) weighing 140 to 170 g, were used. All operations were performed under ether anaesthesia. Renal hypertension was in-

0007-1188/80/030519-06 $01.00

duced by applying a solid silver clip, with an internal diameter of 0.20 mm, to the left renal artery, leaving the contralateral kidney undisturbed (Leenen & De Jong, 1971). Control rats were operated on in the same way, except for the application of the clip. Systolic blood pressure was checked after 15 to 17 days by a tail sphygmographic method, under light ether anaesthesia. The systolic blood pressure and heart rate of the sham-operated rats were 130 ± 2 mmHg and 389 ± 6 beats/min (n = 84) and of the hypertensive rats 189 ± 3 mmHg and 416 ± 6 beats/min (n = 79). A sham operation was performed in the control rats on the same day that the clip was removed from the renal artery of the renal hypertensive rats. These unclipped and control rats were tested 24 h later at which time the unclipped rats were again normotensive (the mean blood pressure and heart rate for unclipped rats were 107 ± 3 mmHg and 396 ± 8 beats/min (n = 58) and for control rats 102 ± 4 mmHg and 371 ± 7 beats/min (n = 58)). In the first three experiments the caudal artery at the base of the tail and the jugular vein were cannulated 18 to 20 h after the removal of the clip or the sham operation. The interval between the cannulation and the intravenous (i.v.) administration of the test substances was 4 to 5 h. The blood pressure was recorded for 30 min before the drugs were given. Nephrectomy, when mentioned was done just before the cannulation, during the same operation session. In the first experiment arachidonic acid (4 mg/kg i.v.) was given to unclipped and control rats. In the second experiment arachidonic acid (2 and 4 mg/kg), bradykinin (4 and 36 μg/kg) and acetylcholine chloride (0.5, 1.6, 5 and 16 μg/kg) were administered (all i.v.) to three separate groups of unclipped and control rats. In the third experiment arachidonic acid (4 mg/kg i.v.) was given to intact or to nephrectomized unclipped and control rats. In the fourth experiment the iliac artery and jugular vein were cannulated immediately after the removal of the clip. Arachidonic acid (4 mg/kg), prostaglandin E_2 (2, 8 and 24 μg/kg) and prostaglandin I_2 (0.5, 2 and 8 μg/kg) were administered intravenously to intact or nephrectomized unclipped and control rats, 24 h after the removal of the clip. The kidneys were removed 4 to 5 h before the intravenous injections.

Estimation of the plasma renin activity

In a separate experiment only the jugular vein of unclipped and control rats was cannulated 18 to 20 h after the removal of the clip. Arachidonic acid (4 mg/kg) or Tris buffer was injected intravenously 4 to 5 h after the cannulation. The rats were replaced in their individual home cages and decapitated 3 min after the injection when the decrease in blood pressure was maximal (Figures 1 and 2). Immediately after decapitation, blood was collected from the trunk for 30 s into plastic tubes containing 10 mg disodium edetate dissolved in 0.2 ml 0.9% w/v NaCl solution (saline). The blood samples were placed on ice and plasma was stored at −20°C. Plasma renin activity was measured according to the method of Haber, Koerner, Page, Kliman & Purnode (1969), using a radioimmunoassay for angiotensin I. Plasma renin activity was calculated as the amount of angiotensin I generated from endogenous substrate per ml of plasma during the incubation of the sample at pH 6.5 for 1 h at 37°C.

Drugs used

Drugs used were: arachidonic acid (Sigma, Chemical Company, St. Louis), which was stored as a stock solution of 10 mg/ml in n-hexane at −20°C. Just before starting the experiment the solvent was evaporated rapidly under nitrogen and the acid was redissolved in 50 mM Tris buffer (pH 7.5) to give the sodium salt. Prostaglandin E_2 (a generous gift of Dr J.E. Pike, Upjohn Company, Michigan) and prostaglandin I_2 (a generous gift of Dr D.N. Whittaker, Wellcome Research Laboratories, Beckenham) were dissolved in 50 mM Tris buffer. Acetylcholine chloride (OPG, the Netherlands), angiotensin II (Hypertensin, a generous gift of Ciba-Geigy, Basel) and bradykinin (a generous gift of Sandoz, Basel) were dissolved in saline. All drugs were dissolved just before the experiment and placed on ice. Drugs were administered as 0.1 ml i.v. bolus injections. Heparin sodium (Leo, Denmark) was dissolved in saline in a concentration of 0.5 mg (80 i.u.)/ml for filling the cannulae.

Statistical methods

The results are expressed as means ± standard error of the mean. Student's t-test was used for statistical analysis of the results. A P value of 0.05 was taken as the criterion of significance.

Results

Arachidonic acid

In a pilot experiment there was a marked difference in the blood pressure response of control and unclipped rats after arachidonic acid (4 mg/kg i.v.). The decrease in blood pressure and change in heart rate were: control rats, −35 ± 10 mmHg and +36 ± 11 beats/min (n = 6) and for unclipped rats, −9 ± 6 mmHg ($P < 0.05$) and +2 ± 8 beats/min ($P < 0.01$) (n = 6). In the experiment in which the iliac artery had been cannulated, there was again a significant difference between the blood pressure decrease of control and of

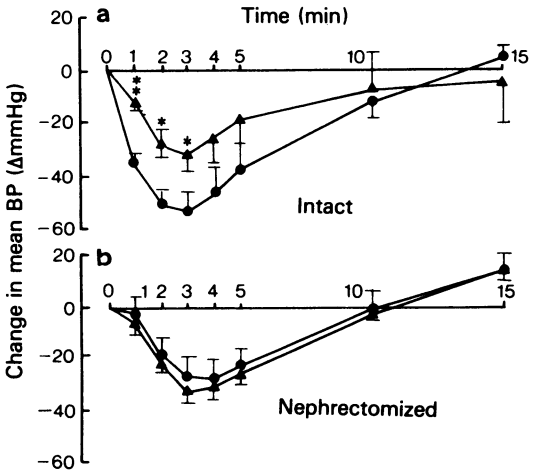

Figure 1 Change in mean blood pressure after injection of arachidonic acid (4 mg/kg i.v.). (▲): Normotensive unclipped rats (renal hypertensive rats with the renal artery clip removed 24 h before the injection); (●): normotensive control rats (normotensive rats sham-operated 24 h before the injection). In (b): unclipped and control rats with both kidneys removed 4 h before the injection; (a): unclipped and control rats sham-operated 4 h before the injection. Data are means from 6 to 9 rats; vertical bars show s.e. mean. * $P < 0.05$; ** $P < 0.01$; levels of significance of the difference between unclipped and control rats.

unclipped rats after intravenous arachidonic acid (Figure 1). The maximal decrease in blood pressure in both groups was reached after 3 min. A tracing of the blood pressure response of control and unclipped rats after arachidonic acid is shown in Figure 2.

Prostaglandins

The maximal decrease in blood pressure was reached sooner after prostaglandin I_2 (Figure 2) than after arachidonic acid. The decrease of blood pressure following different doses of prostaglandin E_2 and I_2 was significantly attenuated in unclipped rats (Figure 3).

Bradykinin and acetylcholine

There was no significant difference in the fall in blood pressure of control and of unclipped rats after various doses of bradykinin or acetylcholine (Figure 4). Arachidonic acid, 4 mg/kg, a dose which decreased blood pressure to the same extent as did the lowest dose of bradykinin and acetylcholine was followed by a significantly attenuated depressor response in unclipped rats.

Nephrectomy

The removal of both kidneys, 4 h before the injection of arachidonic acid (4 mg/kg) abolished the difference in blood pressure response between control and unclipped rats (Figure 5). The depressor response to arachidonic acid was attenuated in the intact unclipped rats (Figure 5). When the iliac artery was cannulated, nephrectomy abolished the difference in blood pressure response of control and of unclipped rats to both arachidonic acid (Figure 1) and prostaglandin E_2 and I_2 (Figure 3).

Plasma renin activity

After arachidonic acid (4 mg/kg) the unclipped rats had a lower plasma renin activity (ng AI ml^{-1} h^{-1}) in peripheral blood than did the control rats (15 ± 2; $n = 12$ vs 28 ± 3; $n = 16$, $P < 0.005$). Similar results followed the administration of vehicle to unclipped rats (11 ± 2; $n = 9$) and control rats (29 ± 5; $n = 10$) ($P < 0.01$).

Discussion

In the experiments described here, the blood pressure of renal hypertensive rats, in which the clip was removed, was decreased by prostaglandins E_2 and I_2 and by the prostaglandin precursor, arachidonic acid. One day after removal of the clip, when rats were again normotensive, the depressor response to arachidonic acid was lessened as was the response to prostaglandins E_2 and I_2. The altered depressor response was thus not the direct result of arachidonic acid conversion being inhibited.

Prostaglandins decrease the blood pressure by lessening the total peripheral resistance in the systemic circulation while having either no apparent negative inotropic effect or a slight positive inotropic effect (Ogletree, Beardsley & Lefer, 1975; Fitzpatrick, Alter, Corey, Ramwell, Rose and Kot, 1978; Armstrong, Dusting, Moncada & Vane, 1978). The diminished response seen in the present experiments may depend on an attenuated reactivity of the resistance vessels to prostaglandins, or on an increased degradation of prostaglandins. This awaits more direct proof. The response to various doses of bradykinin and acetylcholine was not decreased which may suggest that the alteration of the response to hypotensive prostaglandins is an effect which is specific for the prostaglandins.

Removal of both kidneys abolished the difference in decrease in blood pressure of unclipped and of control rats. The kidney therefore seems to play an important role in the attenuation of the depressor response to prostaglandins in unclipped rats. An

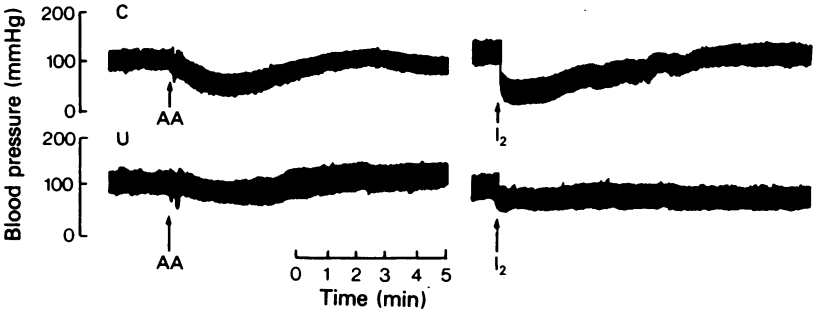

Figure 2 Tracings of blood pressure showing response of unclipped rats (below: U) and control rats (above: C) to intravenous bolus injections of arachidonic acid (4 mg/kg) (left side: AA) or prostaglandin I_2 (8 μg/kg) (right side: I_2).

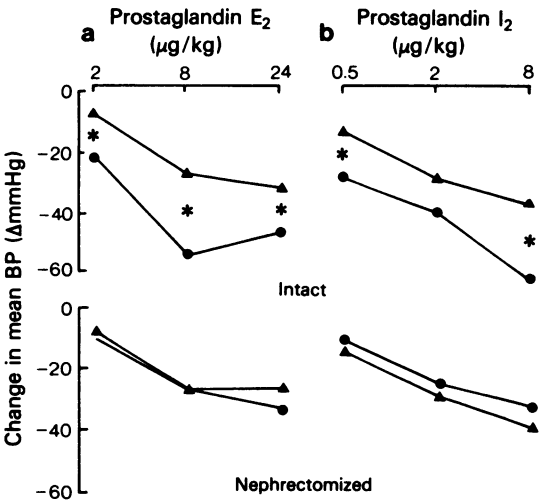

Figure 3 Maximal decrease of mean blood pressure after intravenous injection of prostaglandin E_2 (a) and prostaglandin I_2 (b). (▲): Normotensive unclipped rats (renal hypertensive rats with the renal artery clip removed 24 h before the injection); (●): normotensive control rats (normotensive rats sham-operated 24 h before the injections). Lower figures: unclipped and control rats with both kidneys removed 4 h before the injections. Top figures: unclipped and control rats sham-operated 4 h before the injections. Data are means from 6 to 9 rats. * $P < 0.05$, level of significance between unclipped and control rats.

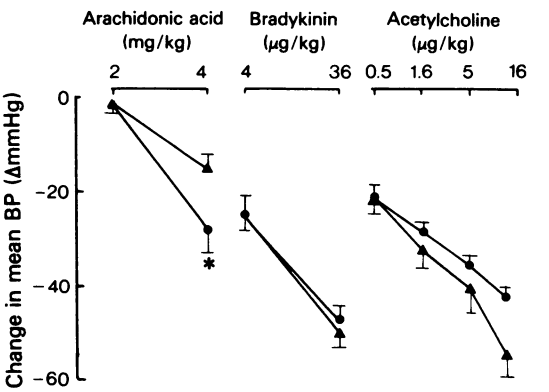

Figure 4 Decrease of mean blood pressure after intravenous injection of arachidonic acid (mg/kg), bradykinin (μg/kg), or acetylcholine (μg/kg). (▲): Normotensive unclipped rats (renal hypertensive rats with the renal artery clip removed 24 h before the injections); (●): normotensive control rats (normotensive rats sham-operated 24 h before the injections). Data are means from 6 to 11 rats; vertical bars show s.e. means. * $P < 0.05$ level of significance of the difference between unclipped and control rats.

enhanced secretion of renin by the kidneys of unclipped rats, induced by the administration of arachidonic acid and prostaglandins, could contribute to the attenuated depressor response. Arachidonic acid and several of its metabolites stimulate the release of renin by the kidney in rats (Weber, Holzgreve, Stephan & Herbst, 1975), in rabbits (Larsson, Weber & Änggård, 1974) and in dogs (Werning, Vetter, Weidmann, Schweikert, Steil & Siegenthaler, 1971; Osborn, Noordewier, Hook & Bailie, 1978). In addition, the renin secreting system of renal hypertensive rats can be more easily stimulated (Leenen, De Jong & De Wied, 1973; Leenen & De Jong, 1975). However, our results have shown that the plasma renin activity of unclipped and of control rats was similar after arachidonic acid or vehicle. The compensatory secretion of renin thus does not seem to be involved in the attenuated response. The renin content of the contralateral kidney and its capacity to secrete renin are markedly reduced 2 weeks after the application of a clip to the left renal artery (Gross, Brunner &

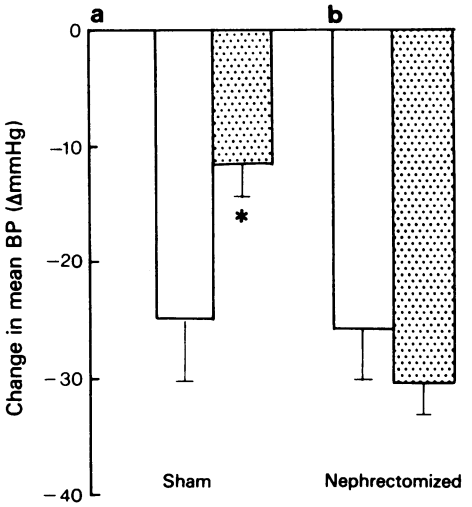

Figure 5 Change in mean blood pressure after injection of arachidonic acid (4 mg/kg i.v.). Stippled columns: normotensive unclipped rats (renal hypertensive rats with the renal artery clip removed 24 h before the injection); open columns: normotensive control rats (normotensive rats sham-operated 24 h before the injection). (a): Unclipped and control rats sham-operated 4 h before the injection; (b): unclipped and control rats with both kidneys removed 4 h before the injection. Data are from 6 rats per group. Vertical bars are s.e. mean. * $P < 0.05$, level of significance of the difference between unclipped and control rats.

Ziegler, 1965; De Jong, 1969). Helmchen & Kneissler (1976) found that plasma renin activity decreased for up to 5 days after removal of the clipped kidney from rats which had been hypertensive for 4 to 6 weeks. The renin secretion of the clipped kidney could be depressed by suddenly increasing the blood pressure in the renal artery, e.g. as occurs after the removal of the clip. Together these factors may explain the diminished plasma renin activity in the peripheral blood of unclipped rats.

The cardiovascular system of unclipped rats has an increased reactivity to renin, angiotensin and noradrenaline; the enhanced pressor response to these agents is an example of this (Skulan, *et al.*, 1974; Ten Berg & De Jong, 1977). The nature of the factor(s) producing the increased reactivity is not entirely understood. Enhanced sensitivity and/or contractility of the resistance vessels may be important factors in the pathogenesis of hypertension (Davis, 1977). A number of vasoconstrictor stimuli can release prostaglandins both *in vivo* and *in vitro*; inhibition of the endogenous synthesis and release of prostaglandins potentiates the vasoconstrictor responses to these stimuli (Messina, *et al.*, 1976). It is tempting to suggest that a decreased reactivity of the cardiovascular system to and/or a diminished availability of prostaglandins is involved in the enhanced blood pressure response of unclipped rats. The effect of nephrectomy raises the possibility that the decreased reactivity and/or diminished availability is related to factor(s) of renal origin.

References

ARMSTRONG, J.M., DUSTING, G.J., MONCADA, S. & VANE, J.R. (1978). Cardiovascular actions of prostacyclin (PGI_2), a metabolite of arachidonic acid which is synthesized by blood vessels. *Circulation Res.*, **(Supp. I) 43,** 112–119.

DAVIS, J.O. (1977). The pathogenesis of chronic renovascular hypertension. *Circulation Res.* **40,** 439–444.

DE JONG, W. (1969). Release of renin by rat kidney slices; relationship to plasma renin after desoxycorticosterone and renal hypertension. *Proc. Soc. exp. Biol. Med.*, **130,** 85–88.

DUNN, M.J. & HOOD, V.L. (1977). Prostaglandins and the kidney. *Am. J. Physiol.*, **233,** F169-F184.

FITZPATRICK, T.M., ALTER, I., COREY, E.J., RAMWELL, P.W., ROSE, J.C. & KOT, P.A. (1978). Cardiovascular responses to PGI_2 (Prostacyclin) in the dog. *Circulation Res.*, **42,** 192–194.

GROSS, F., BRUNNER, H. & ZIEGLER, M. (1965). Renin-angiotensin system, aldosterone, and sodium balance. *Rec. Prog. Horm. Res.*, **21,** 119–167.

HABER, E., KOERNER, T., PAGE, L.B., KLIMAN, B. & PURNODE, A. (1969). Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J. clin. Endocr.*, **29,** 1349–1355.

HAUX, F., INGEROWSKI, R.M. & VON BRUCHHAUSEN, F. (1976). Prostaglandin synthetase activity in kidney medulla during the development of experimental hypertension in the rat. *Naunyn Schmiedebergs Arch. Pharmac.*, **295,** 77–79.

HELMCHEN, U. & KNEISSLER, U. (1976). Role of the renin-angiotensin system in renal hypertension. An experimental approach. *Curr. Top. Pathol.*, **61,** 203–238.

KALEY, G. (1976). The role of prostaglandins in vascular homeostasis. *Fedn Proc.*, **35,** 2358–2359.

LARSSON, C., WEBER, P. & ÄNGGÅRD, E. (1974). Arachidonic acid increases and indomethacin decreases plasma renin activity in the rabbit. *Eur. J. Pharmac.*, **28,** 391–394.

LEARY, W.P., LEDINGHAM, J.G. & VANE, J.R. (1974). Impaired prostaglandin release from the kidneys of salt-loaded and hypertensive rats. *Prostaglandins*, **10,** 425–432.

LEENEN, F.H.H. & DE JONG, W. (1971). A solid silver clip for induction of predictable levels of renal hypertension in the rat. *J. appl. Physiol.*, **31,** 142–144.

LEENEN, F.H.H., DE JONG, W. & DE WIED, D. (1973). Renal venous and peripheral plasma renin activity in renal hypertension in the rat. *Am. J. Physiol.*, **225,** 1513–1518.

LEENEN, F.H.H. & DE JONG, W. (1975). Plasma renin and sodium balance during development of moderate and severe renal hypertension in rats. *Circulation Res.*, **36–37 (Suppl. I),** 179–186.

MCGIFF, J.C., MALIK, K.U. & TERRAGNO, N.A. (1976). Prostaglandins as determinants of vascular reactivity. *Fedn Proc.*, **35,** 2382–2387.

MESSINA, E.J., WEINER, R. & KALEY, G. (1976). Prostaglandins and local circulatory control. *Fedn Proc.*, **35,** 2367–2375.

NIJKAMP, F.P. & DE JONG, W. (1978). Possible role of prostaglandins in the development of renal hypertension in the rat. *7th Int. Congress of Pharmac., Paris, July 16–21,* Abstract no. **2494,** p. 805.

OGLETREE, M.L., BEARDSLEY, A.C. & LEFER, M. (1975). Myocardial actions of prostaglandins in isolated cat cardiac tissue. *Life Sci. Oxford,* **16,** 1923–1930.

OSBORN, J.L., NOORDEWIER, B., HOOK, J.B. & BAILIE, M.D. (1978). Mechanism of prostaglandin E_2 stimulation of renin secretion. *Proc. Soc. exp. Biol. Med.*, **159,** 249–252.

PAMNANI, M.B., SIMON, G. & OVERBECK, H.W. (1976). Bioassay *in vivo* for circulating vasoactive agents after renal artery constriction in dogs. *Circulation Res.*, **39,** 517–523.

PUGSLEY, D.J., BEILIN, L.J. & PETO, R. (1975). Renal prostaglandin synthesis in the Goldblatt hypertensive rat. *Circulation Res.* **(Suppl. I), 36–37,** 81–88.

ROMERO, J.C. & STRONG, C.G. (1977). The effect of indomethacin blockade of prostaglandin synthesis on blood pressure of normal rabbits and rabbits with renovascular hypertension. *Circulation Res.*, **40,** 35–41.

SCHÖLKENS, B.A. & STEINBACH, R. (1975). Increase of experimental hypertension following inhibition of prostaglandin biosynthesis. *Archs int. Pharmacodyn. Ther.*, **214,** 328–334.

SIROIS, P. & GAGNON, D.J. (1974). Release of renomedullary prostaglandins in normal and hypertensive rats. *Experientia,* **30,** 1418–1419.

SKULAN, T.W., BROUSSEAU, A.C. & LEONARD, K.A. (1974). Accelerated induction of two-kidney hypertension in rats and renin-angiotensin sensitivity. *Circulation Res.*, **35,** 734–741.

SMITH, G.W. & SOMOVA, L.I. (1976). Renal function and renal venous prostaglandin concentrations during different stages of experimental renal hypertension in the rat. *Br. J. Pharmac.*, **58,** 253–259.

TEN BERG, R.G.M., LEENEN, F.H.H. & DE JONG, W. (1979). Plasma renin activity and sodium, potassium, water excretion during reversal of hypertension in one-clip, two-kidney hypertensive rats. *Clin. Sci. Mol. Med.*, **57,** 47–52.

TEN BERG, R.G.M. & DE JONG, W. (1977). Enhanced reactivity of blood pressure to reapplication of a renal artery clip in unclipped renal hypertensive rats. *Proceedings of the 27th Int. Congress of Physiol. Sci., July 18–23, Paris,* abstract no. **2226,** p. 748.

VANE, J.R. & MCGIFF, J.C. (1975). Possible contributions of endogenous prostaglandins to the control of blood pressure. *Circulation Res.*, **(Suppl. I), 36–37,** 68–75.

WEBER, P., HOLZGREVE, H., STEPHAN, R. & HERBST, R. (1975). Plasma renin activity and renal sodium and water excretion following infusion of arachidonic acid in rats. *Eur. J. Pharmac.*, **34,** 299–304.

WERNING, C., VETTER, W., WEIDMANN, P., SCHWEIKERT, H.U., STEIL, D. & SIEGENTHALER, W. (1971). Effect of prostaglandin E_1 on renin in the dog. *Am. J. Physiol.*, **220,** 852–856.

(Received April 2, 1979.)