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RELEASE OF PROSTAGLANDINS FROM THE RABBIT PERFUSED KIDNEY: EFFECTS OF VASOCONSTRICTORS

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1 The rat stomach strip was used to assay prostaglandin E_2-like material released by a rabbit isolated kidney perfused with Krebs solution.

2 Doses of noradrenaline and angiotensin II producing similar vasoconstrictor effects released equivalent amounts of prostaglandins from the kidney.

3 8-Leu-angiotensin II, a specific inhibitor of the natural octapeptide, blocked the action of angiotensin II on perfusion pressure and the release of prostaglandins, while the action of noradrenaline on both parameters was unaffected.

4 Indomethacin, a specific inhibitor of prostaglandin biosynthesis, blocked the effects of both vasoconstrictors on prostaglandin release while their action on perfusion pressure was significantly enhanced.

5 In the kidney effluent, amounts of prostaglandin E_2-like material increased linearly with the rise in perfusion pressure induced by increasing doses of angiotensin II. These results indicate that prostaglandin output from the isolated kidney follows the rise in perfusion pressure.

Introduction

Numerous studies *in vivo* have demonstrated that prostaglandins can be released from the kidney by various procedures which alter the vascular resistance of that organ. Renal nerve stimulation, noradrenaline (Dunham & Zimmerman, 1970; McGiff, Crowshaw, Terragno, Malik & Lonigro, 1972) and angiotensin II (McGiff, Crowshaw, Terragno & Lonigro, 1970) increased prostaglandin E-like material in the renal vein of the dog. Blockade of prostaglandin output by indomethacin has recently been described by Davis & Horton (1972) in the rabbit kidney.

Furthermore, in the isolated spleen, Douglas, Johnson, Marshall, Jaffe & Needleman (1973) have shown that specific inhibitors of angiotensin and noradrenaline antagonize the release of prostaglandin-like material induced by both vasoconstrictors.

The present work was designed to study: (a) the effects of vascular changes induced by noradrenaline and angiotensin II on the release of prostaglandins by the rabbit isolated perfused kidney; (b) the effects of 8-Leu-angiotensin II, a specific inhibitor of angiotensin II (Regoli, Park, Rioux & Chan, 1971) and of indomethacin, an inhibitor of the biosynthesis of prostaglandins (Ferreira, Moncada & Vane, 1971); and (c) the relationship between the extent of vasoconstriction and the amount of prostaglandin-like material released from the kidney.

Methods

Isolated kidney

New Zealand rabbits of either sex weighing between 1.3 to 2 kg were killed by a stunning blow. The left kidney was rapidly excised and the renal artery cannulated with polyethylene tubing (PE 190).

The kidney was placed in a small polyethylene chamber and perfused immediately at a constant rate of 5 ml/min with Krebs solution maintained at 37° C. A pressure transducer was connected to a side-arm of the arterial cannula and the perfusion pressure was continuously recorded on a Hellige polygraph. The Krebs solution, gassed continuously with 95% O_2 and 5% CO_2, had the following composition (g/l): NaCl, 6.9; KCl, 0.35; $CaCl_2$, 0.28; KH_2PO_4, 0.16; $MgSO_4 .7H_2O$, 0.29; dextrose, 2; $NaHCO_3$, 2.1.

Bioassay

Rats of either sex were killed by a stunning blow and bled through the carotid arteries. For each experiment, two rat stomach strips were prepared in cooled Krebs solution according to Vane (1957). The strips were mounted in an organ bath and immediately superfused at a rate of 5 ml/min with oxygenated Krebs solution at 37°C as described above.

The technique of superfusion was basically that of Gaddum (1953): the tissues were counterweighted with 1.5-2 g, and their movements were recorded with a Harvard Heart and Smooth Muscle transducer, model No. 356, connected to an electronic Harvard recording system.

To obtain specific responses of the assay organs to prostaglandins, a mixture of inhibitors containing (μg/ml): methysergide, 0.2; phenoxybenzamine, 0.1; propranolol, 3.0; atropine, 0.1; diphenhydramine, 0.1, was infused (0.1 ml/min) into the renal effluent. Under such conditions, the rat stomach strip contracts to amounts of prostaglandin E_2 as low as 1 ng (Higgs & Youlten, 1972). When angiotensin II was infused through the kidney, 8-Leu-angiotensin II (100 ng/ml) was added to the mixture of inhibitors to prevent any direct action of the octapeptide upon the assay organs.

After a renal washout period of 30 min, the Krebs solution superfusing the rat stomach strips was replaced by the kidney effluent for the remainder of the experiment. Once the tissues had stablized, a dose-response curve was obtained with infusion of prostaglandin E_2 (1, 3, 5 and 10 ng/ml) into the fluid leaving the kidney. The contractions of the assay organs were measured in centimetres.

Results are expressed as mean ± s.e. and significance of differences has been calculated with the *t*-test for paired data. Correlation coefficient and significance were calculated according to the method of Steel & Torrie (1960).

The following drugs were used: angiotensin II amide (Ciba Co.); indomethacin-lactose (Merck Frosst Laboratories); 8-Leu-angiotensin II (synthesized by Dr W.K. Park in our department); atropine sulphate; (−)-arterenol bitartrate (Sigma Chemicals Co.); phenoxybenzamine hydrochloride (Smith Kline & French Co.); diphenhydramine hydrochloride (Parke Davis Co.); (−)-propranolol hydrochloride (Ayerst Laboratories); methysergide bimaleate (Sandoz Co.); and prostaglandin E_2 (ONO Pharmaceuticals).

All drugs were freshly prepared in Krebs solution, and ascorbic acid was added to solutions of noradrenaline. Angiotensin II, noradrenaline, indomethacin and 8-Leu-angiotensin II were infused through a polyethylene tube in the perfusing fluid at a rate of 0.1 ml/minute.

Results

In the first series of experiments, we studied the release of renal prostaglandins induced by doses of angiotensin II (30 ng/ml) and noradrenaline (50 ng/ml) which, under our experimental conditions, produced equal pressure rises at a constant flow (5 ml/minute). The effects produced by both vasoconstrictors were investigated in the presence and the absence of either indomethacin or 8-Leu-angiotensin II.

Effects of 8-Leu-angiotensin II

In these experiments, 8-Leu-angiotensin II was continuously infused either directly over the assay organs to prevent any direct action of angiotensin II upon the tissues, or through the kidney in order to prevent the actions of the natural octapeptide upon the perfusion pressure and the assay organs. The antagonist did not have any intrinsic action upon the parameters studied.

In the first series of experiments, angiotensin II (30 ng/ml) and noradrenaline (50 ng/ml) elicited equal pressure rises and equal increases of the renal output of prostaglandins of 3.13 ± 0.25 and 3.26 ± 0.36 (ng/ml) respectively, evaluated as prostaglandin E_2-like activity. The infusions of both vasoconstrictors were repeated 30 min after the beginning of a continuous infusion of 8-Leu-angiotensin II through the kidney. In the presence of the antagonist, the action of angiotensin II upon the perfusion pressure as well as on the release of prostaglandins was then completely inhibited (Fig. 1) while noradrenaline induced changes in perfusion pressure which were even greater than those observed before the inhibitor, and the increase in prostaglandin release was significantly greater ($P < 0.05$).

Effects of indomethacin

In order to obtain more information on the spontaneous release of prostaglandins by the kidney, the following protocol was designed: a dose-response curve to prostaglandin E_2 (1, 3, 5 ng/ml) was recorded by injecting the agonist directly to the tissues, treated with 2 μg/ml indomethacin. Thereafter, the Krebs solution was replaced with the kidney effluent; the base-line of the tissue increased; expressed as prostaglandin E_2-like activity, the contraction corresponded to that given by 0.82 ng/ml of E_2, which probably

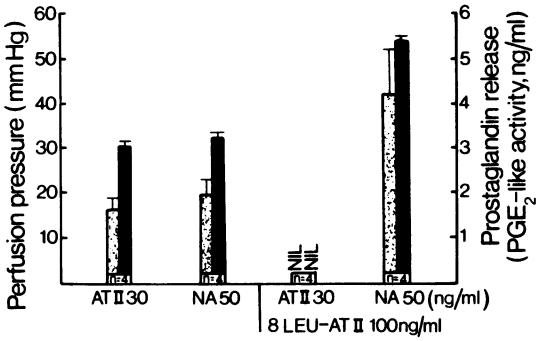

Fig. 1 Effects of angiotensin II (AT_{II}) and noradrenaline (NA) upon the perfusion pressure (stippled columns) and prostaglandin output (PGE_2-like activity) (solid columns) from the isolated perfused kidney of the rabbit, before and during the continuous infusion of 8-Leu-angiotensin II (8-Leu-AT_{II}) into the renal circulation. *n* = number of experiments.

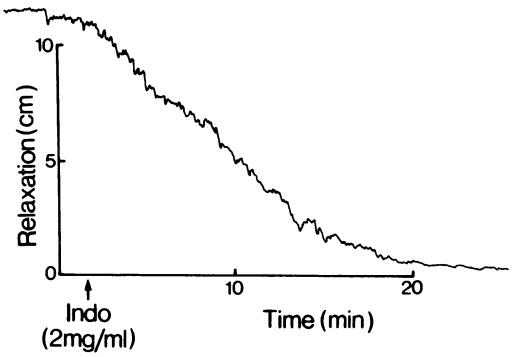

Fig. 2 Typical relaxation of a rat stomach strip superfused with the effluent from the isolated perfused (5 ml/min, at 37° C) kidney of the rabbit, during the continuous infusion of indomethacin into the renal circulation.

represents the spontaneous secretion of prostaglandins from the isolated kidney.

The base-line remained at this new level and the effect of exogenous prostaglandin E_2 applied directly to the tissue was initially reduced by about 15%. The sensitivity to exogenous prostaglandin E_2 was further decreased after 1 h of infusion, indicating the endogenous prostaglandins desensitized the rat stomach strips to prostaglandin E_2.

Further evidence that the contraction produced by the kidney effluent was due to prostaglandins emerged from the experiment with indomethacin. Figure 2 shows the effect of indomethacin infused into the kidney. By blocking the prostaglandin release with indomethacin, the base-line of rat stomach strips decreased. In addition, the sensitivity of the rat stomach strips to exogenous prostaglandins was restored to the initial levels. The effect of angiotensin II and noradrenaline in kidney treated with indomethacin was studied by infusing for 10 min both vasoconstrictors before and during the administration of indomethacin (2 μg/ml) to the kidney.

As shown in Fig. 3, angiotensin II (30 ng/ml) and noradrenaline (50 ng/ml) induced equivalent increases in perfusion pressure which reached the maximum after 3 and 4 min respectively: thereafter, the perfusion pressure decreased slowly.

In these experiments, the prostaglandin levels in the renal effluent started to increase after the vascular changes had occurred that is about 3-4 min after the beginning of the infusion. Then, prostaglandin levels reached a plateau and diminished gradually 7 and 8 min later.

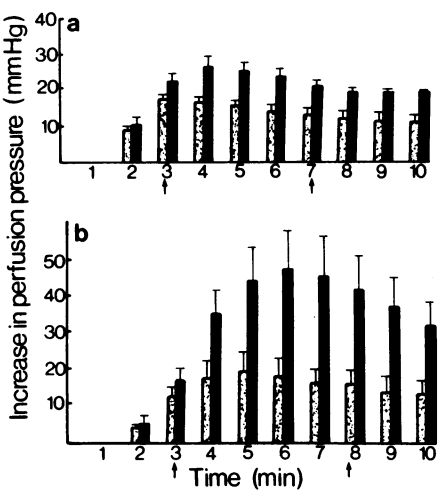

Fig. 3 Effects of continuous (10 min) infusions of (a) angiotensin II (AT_{II}) 30 ng/ml and (b) noradrenaline (NA) 50 ng/ml upon the perfusion pressure of the isolated perfused kidney of the rabbit before (stippled columns) and during (solid columns) the infusion of indomethacin. Arrows show the time at which the level of prostaglandins increased over and started to decrease towards control values. Vertical bars represent standard error of the means. Number of experiments = 7.

Infusions of both vasoconstrictors were performed before and 45 min after continuous administration of indomethacin (2 $\mu g\ ml^{-1}\ min^{-1}$) to the kidney. The infusion of indomethacin had no effect on the basal level of the perfusion pressure (50 mmHg). In the presence of indomethacin, the vascular changes induced by both

vasoconstrictors occurred without prostaglandin release. Furthermore, after their vascular effects had fully developed, the increase in perfusion pressure induced by angiotensin II and noradrenaline were greater ($P < 0.05$) than those observed in the absence of indomethacin (Figure 3).

It was observed that the changes in vascular response to noradrenaline were greater than those to angiotensin II when the biosynthesis and consequently, the release of prostaglandins, were inhibited by indomethacin.

In view of these findings, we wanted to investigate the possible relationship between the concentration of angiotensin II in the renal circulation, the degree of vascular change and the amount of prostaglandins released by the kidney. Thus, in the next series of experiments, the effects of increasing doses (3, 10 and 30 ng/ml) of angiotensin II were investigated in 12 preparations.

Figure 4a shows that the various doses of angiotensin II induced dose-dependent rises of the basal output of prostaglandins from the kidney. As shown in the figure, the doses of angiotensin II (3, 10 and 30 ng/ml) released 1.33 ± 0.28, 2.60 ± 0.37 and 4.15 ± 0.36 ng/ml prostaglandins (as prostaglandin E_2-like activity), respectively. These values were significantly different from each other. There was a highly significant correlation ($P < 0.001$) between the doses of angiotensin II and the increase in the basal output of prostaglandins from the kidney.

Similarly, the various doses of angiotensin II produced dose-dependent increases in perfusing pressure (3.42 ± 0.34, 8.00 ± 1.13 and 15.28 ± 1.68 mmHg, respectively) as shown in Figure 4b. These vascular changes were again, significantly different from each other. As can be seen in the figure, there was a highly significant correlation between the doses of angiotensin II and the degree of vascular changes.

Since we have shown in Figs. 1 and 3 that the vascular effect and the prostaglandin release are related phenomena and that the vascular effect occurred before the release of prostaglandins started to rise, we evaluated the correlation between the degree of the rise in perfusion pressure induced by the various doses of angiotensin II and the increase in the output of prostaglandins from the kidney. As shown in Fig. 4c, a highly significant value ($P < 0.001$) was obtained.

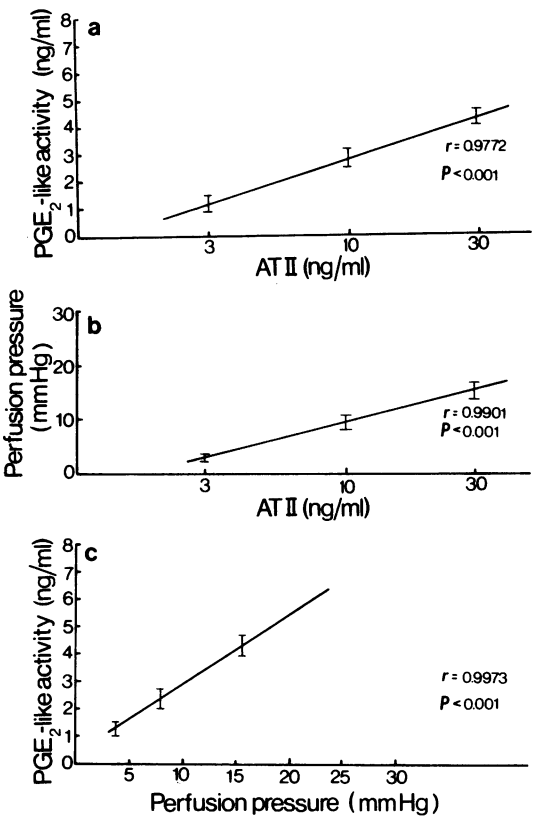

Fig. 4 Effects of infusion of increasing concentrations of angiotensin II (AT_{II}) into a perfused isolated kidney of the rabbit upon (a) the basal prostaglandin (PGE_2-like activity) output and (b) the perfusion pressure. (c) Shows the correlation between both parameters. Number of experiments = 12; r = correlation coefficient; P = degree of significance.

Discussion

The administration of angiotensin II and noradrenaline produced increases in perfusion pressure and in the basal output of prostaglandin-like material from the perfused kidney, thus confirming previous observations by Dunham & Zimmerman (1970).

Our results clearly show that the vascular effects and the increase in prostaglandin release induced by infusions of angiotensin II and noradrenaline are related phenomena and that the vascular changes occurred before the prostaglandin output started to rise.

Our results also showed that following the administration of indomethacin in the renal circulation, the assay organs relaxed gradually over a period of 20 minutes. This is consistent with the observations that in conditions where assay tissues are superfused with the effluent of a prostaglandin-synthesizing organ, the basal output of the prostaglandins contributes to the overall muscle

tone and possibly to a partial desensitization of the tissue. Using an isolated spleen preparation Ferreira *et al.* (1971) observed similar effects after indomethacin and they inferred a decrease in basal secretion of prostaglandins due to the inhibition of their biosynthesis by indomethacin. In the presence of indomethacin, the isolated kidney released basal amounts of prostaglandin E_2 that were not detectable by the assay method used which had a sensitivity of 0.5 to 1.0 ng/ml of prostaglandin E_2.

Furthermore, in the presence of indomethacin, the effects of angiotensin II and noradrenaline on the perfusion pressure were greatly augmented. These results would suggest that since prostaglandins were no longer available to oppose their vascular actions, angiotensin and noradrenaline had a greater action on the rabbit kidney vascular bed. These results confirm that prostaglandins may be involved in the process of autoregulation by the kidney, as suggested by Herbaczynska-Cedro & Vane (1973).

However, using agents that have equivalent actions on perfusion pressure and consequently release the same amounts of prostaglandins, we would expect that in the presence of indomethacin, the increase in vascular responses would be of the same degree. Our results show that the vascular changes induced by angiotensin II, although significant, were less enhanced than those induced by noradrenaline. These differences may be due to a different site of action of these agents upon the kidney vascular bed (Regoli & Gauthier, 1971). However, the possibility that angiotensin may liberate other vasodilator agents which are not blocked by indomethacin cannot be ruled out. This point will need further investigation.

The administration of 8-Leu-angiotensin II, a specific inhibitor of the natural octapeptide (Regoli *et al.*, 1971) completely prevented the vascular changes normally induced by angiotensin as well as the subsequent changes in basal secretion of prostaglandins. Although we did not try to prevent the action of noradrenaline on both parameters in our preparation, recent findings of Douglas *et al.* (1973) showed that these could be prevented by the use of phenoxybenzamine. In the presence of a dose of 8-Leu-angiotensin II capable of blocking the actions of angiotensin II, the vascular changes and, especially, the release of prostaglandins induced by noradrenaline were potentiated. The present knowledge of the physiological actions of 8-Leu-angiotensin II does not permit us to draw any conclusion about this observation.

As previously demonstrated by Dunham & Zimmerman (1970), prostaglandin-like material is released from the dog kidney during an increase in renal vascular resistance. Furthermore, in the dog, McGiff *et al.* (1970) observed a correlation between the rise in renal vascular resistance and the dose of angiotensin II. However, the same authors could not demonstrate a relationship between the release of prostaglandin-like substance and the dose of angiotensin II or the degree of renal vasoconstriction (McGiff, Crowshaw, Terragno & Lonigro, 1971). Recently, Aiken & Vane (1973) showed the relationship between the release of prostaglandins and the dose of angiotensin II. Our last series of experiments confirmed this point. The present results suggest the existence of a direct correlation between the extent of the vasoconstriction and the amount of prostaglandins released by the kidney (see Figure 4c). In addition, the appearance of prostaglandins in the renal effluent does not occur simultaneously with the pressor effect, but 1 or 2 min later. It is tempting to postulate a cause-effect relationship between the vasoconstriction and the prostaglandin release. Further evidence in favour of this hypothesis emerges from a series of experiments in which angiotensin II and noradrenaline were used to stimulate the release of prostaglandins from the incubated rabbit renal papilla (unpublished results). Neither angiotensin II nor noradrenaline stimulated the release of prostaglandins although the renal papilla is thought to be the main source of prostaglandins in the kidney (Daniels, Hinman, Leach & Muirhead, 1967; Van Dorp, 1971). In an attempt to distinguish two possible different receptors for angiotensin II, mediating the vasoconstriction and the prostaglandin release separately, we used 8-Leu-angiotensin II. The antagonist abolished both the vasoconstriction and the prostaglandin release induced by angiotensin II, without affecting the effects of noradrenaline. In conclusion, these data show an apparent correlation between the vasoconstriction and the release of prostaglandins. However, the possibility cannot yet be excluded that the two phenomena depend on the stimulation of two different receptors. Further investigation is needed to establish this point.

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References

AIKEN, J.W. & VANE, J.R. (1973). Intrarenal prostaglandin release attenuates the renal vasoconstrictor activity of angiotensin. *J. Pharmac. exp. Ther.*, **184**, 678-687.

DANIELS, E.G., HINMAN, J.W., LEACH, B.E. & MUIRHEAD, E.E. (1967). Identification of prostaglandin E_2 as the principal vasodepressor lipid of rabbit renal medulla. *Nature, Lond.*, **215**, 1298-1299.

DAVIS, H.A. & HORTON, E.W. (1972). Output of prostaglandins from the rabbit kidney, its increase on renal nerve stimulation and its inhibition by indomethacin. *Br. J. Pharmac.*, **46**, 658-675.

DOUGLAS, J.R. Jr, JOHNSON, E.M. Jr, MARSHALL, G.R., JAFFE, B.M. & NEEDLEMAN, P. (1973). Stimulation of splenic prostaglandin release by angiotensin and specific inhibition by cysteine^8-AT_{II}. *Prost.*, **3**, 67-73.

DUNHAM, E.W. & ZIMMERMAN, B.G. (1970). Release of prostaglandin-like material from dog kidney during nerve stimulation. *Amer. J. Physiol.*, **219**, 1279-1285.

FERREIRA, S.H., MONCADA, S. & VANE, J.R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature, New Biol.*, **231**, 237-239.

GADDUM, J.H. (1953). The technique of superfusion. *Br. J. Pharmac. Chemother.*, **8**, 321-326.

HERBACZYNSKA-CEDRO, K. & VANE, J.R. (1973). An intra-renal role for prostaglandin production. Abstracts of *Seventh Annual Meeting of European Society for Clinical Investigation*, Rotterdam.

HIGGS, G.A. & YOULTEN, L.J.F. (1972). Prostaglandins production by rabbit peritoneal polymorphonuclear leukocytes *in vitro*. *Br. J. Pharmac.*, **44**, 330P.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A. & LONIGRO, A.J. (1970). Release of a prostaglandin-like substance into renal venous blood in response to angiotensin II. *Circ. Res.*, suppl. **26**, 121-130.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A. & LONIGRO, A.J. (1971). Renal prostaglandins: Their biosynthesis, release, effects and fate. In: *Renal Pharmacology*, pp. 211-240. N.Y., Appleton, Century Crofts.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A., MALIK, K.U. & LONIGRO, A.J. (1972). Differential effect of noradrenaline and renal nerve stimulation on vascular resistance in the dog kidney and the release of a prostaglandin E-like substance. *Clin. Sc.*, **42**, 223-233.

REGOLI, D. & GAUTHIER, R. (1971). Site of action of angiotensin and other vasoconstrictors on the kidney. *Can. J. Physiol. Pharmac.*, **49**, 608-612.

REGOLI, D., PARK, W.K., RIOUX, F. & CHAN, C.S. (1971). Antagonists of angiotensin, substitution of an aliphatic chain to phenyl ring in position 8. *Rev. Can. Biol.*, **30**, 319-329.

STEEL, R.G.D. & TORRIE, J.H. (1960). *Principles and procedures of statistics.* N.Y., Toronto, London, McGraw-Hill Book Co. Inc.

VAN DORP, D. (1971). Recent developments in the biosynthesis and the analysis of prostaglandins. *N.Y. Acad. Sci.*, **180**, 181-199.

VANE, J.R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344-349.

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