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EVIDENCE FOR PROSTAGLANDIN MEDIATED PREJUNCTIONAL CONTROL OF RENAL SYMPATHETIC TRANSMITTER RELEASE AND VASCULAR TONE

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1 Prostaglandin E_2 dose-dependently and reversibly inhibited the noradrenaline overflow resulting from nerve stimulation of the rabbit kidney.

2 The magnitude of this inhibition varied inversely with the frequency of stimulation employed.

3 The prostaglandin synthesis inhibitors, indomethacin and meclofenamic acid, both increased the transmitter overflow resulting from renal nerve stimulation, suggesting that endogenous prostaglandin has a role in the regulation of transmitter release.

4 In the presence of indomethacin, the inhibitory effect of exogenous prostaglandin E_2 was enhanced.

5 The prostaglandin precursor, arachidonic acid, also caused a significant, dose-dependent and reversible inhibition of transmitter overflow. This inhibition became insignificant when arachidonic acid was applied in the presence of indomethacin, suggesting that the inhibition was mediated by newly formed prostaglandin rather than by arachidonic acid itself.

6 It is proposed that newly formed prostaglandin controls noradrenaline release primarily from inner cortical nerve endings, thereby maintaining juxtamedullary blood flow under periods of increased sympathetic nerve activity.

Introduction

The rabbit kidney has a high capacity for both synthesis (predominantly in the medulla) and degradation (in the renal cortex) of prostaglandins (Hamberg, 1969; Crowshaw, 1971; Larsson & Änggård, 1973). Prostaglandin E_2 is the most abundant renal prostaglandin and is a potent vasodilator (Lee, Crowshaw, Takman, Attrep & Gougoutas, 1967; Daniels, Hinman, Leach & Muirhead, 1967). Consequently, it has been postulated that this prostaglandin has a physiological role in blood flow regulation in the kidney (McGiff, Crowshaw, Terragno & Lonigro, 1970; Lonigro, Terragno, Malik & McGiff, 1973; McGiff & Itskovitz, 1973; Herbaczynska-Cedro & Vane, 1973, 1974; Larsson & Änggård, 1974; Needleman, Douglas, Jalsetik, Stoecklein & Johnson, 1974). It has in addition been shown that both renal nerve stimulation (RNS) and catecholamine administration increase the output of prostaglandins in the renal venous effluent of rabbits, prostaglandin E_2 being most abundant (Davis & Horton, 1972; Needleman *et al.*, 1974).

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In the light of previous evidence which suggests a role for prostaglandin E_2 in regulation of transmitter release from sympathetic nerve endings (Hedqvist, 1970, 1973, 1974a, b), it was decided to examine the possibility of such an interaction in the rabbit kidney. Previous experiments have shown that prostaglandin E_2 has an inhibitory effect on vascular responses of the rabbit kidney to RNS *in vitro* and *in situ* and that this inhibition is probably, in part, prejunctional (Frame, Hedqvist & Åström, 1974; Frame & Hedqvist, 1974).

The aim of the present study was to assess the capacity of prostaglandin E_2, prostaglandin precursor (arachidonic acid) and prostaglandin synthesis inhibitors (indomethacin, meclofenamic acid) to affect transmitter overflow resulting from RNS.

Methods

Forty rabbits weighing 2 to 3 kg were anaesthetized with 25% urethane, 7 ml/kg. The abdomen was opened along its midline and the left

kidney, with its nervous and vascular supply, was dissected free from the surrounding tissue. The nerve was then carefully freed from the artery. After heparinization (1000 i.u./kg, i.v.) the vessels and ureter were cannulated, the nerve was cut and the kidney was flushed with 0.9% w/v NaCl solution (saline) containing 50 i.u. of heparin per ml. The preparation was then transferred to a perfusion chamber maintained at 37°C and was perfused with Tyrode solution (mM: NaCl 136.7, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.5, ascorbic acid 0.1) also at 37°C, containing 2% dextran and gassed with 5% CO_2 in O_2.

The renal perfusion rate was kept constant at 10 ml/min and pressure was recorded on a Grass Model 5 polygraph using a Statham P 23 AC pressure transducer.

Noradrenaline (NA) stores were labelled by infusion of 25-50 μCi of tritiated NA in Tyrode solution over a period of about 20 minutes. The kidney was then perfused with isotope-free Tyrode solution for 30 min to remove surplus tritiated NA.

The nerve was placed on platinum electrodes and stimulated with a Grass S5 stimulator delivering 15 to 75 s trains of pulses (2-10 Hz, 2 ms, supramaximal voltage) at 10 min intervals. The total number of pulses applied per stimulation period was kept constant regardless of frequency. The perfusate was collected in 1 min fractions for 2 min prior to nerve stimulation, during stimulation and for 2 min post-stimulation. The tritium content of the perfusate was determined by counting 0.5 ml aliquots in a Packard liquid scintillation spectrometer with quenching monitored by internal standards. Twenty ml of a 3 : 7 ethanol-toluene mixture containing 4 g of 2-5 diphenyloxazole (PPO) and 0.1 g of 1-4 di (2-(4-methyl-5-phenyloxazolyl))benzene (POPOP) per litre of toluene was used as a counting medium for each sample.

The radioactivity of the perfusate samples was separated into that of intact NA and that of its metabolites by cation exchange column chromatography (Fredholm & Hedqvist, 1973). The recovery of authentic NA added to the samples and carried through the entirely chromatographic procedure was 73.3 ± 2.3% (mean ± s.e. mean, $n = 4$).

The following drugs were used: arachidonic acid, Sigma Chemicals, St. Louis, U.S.A.; [^{3}H]-(−)-noradrenaline, 7.2 Ci/mmol, The Radiochemical Centre, Amersham; indomethacin was a gift from Merck, Sharpe & Dohme, Rahway, N.J., U.S.A.; meclofenamic acid from Parke Davies, Pontypool, Wales, and prostaglandin E_2 from Dr J. Pike, Upjohn Co., Kalamazoo, Mich., U.S.A.

Arachidonic acid (99%, porcine liver) was further purified by silicic acid column chromatography shortly before use. All drugs were administered close-arterially to the kidney in Tyrode or saline solution.

Results

Stimulation of the renal nerves (2-10 Hz, 2 ms duration, supramaximal voltage) caused a marked increase in overflow of radioactivity from the kidney, which was accompanied by an increase in the perfusion pressure. During RNS, intact [^{3}H]-NA accounted for 81.3% of the radioactivity, compared to 14.4% during resting periods. In contrast, material not adsorbed to the column (mainly deaminated products) accounted for only 16.8% during RNS but 74.4% during resting periods. The content of [^{3}H]-normetanephrine, which made up 10% of the radioactivity during rest decreased to less than 2% during stimulation (Table 1).

Effect of exogenous prostaglandin E_2

It was consistently found that infusions of prostaglandin E_2 ($1.8\text{-}9.1 \times 10^{-7}$M) 2 min prior to and during RNS, which did not alter the basal renal arterial pressure, decreased the overflow of transmitter in response to RNS in a dose-dependent manner (Figure 1). The degree of inhibition of [^{3}H]-NA overflow varied inversely

Table 1 Cation exchange column chromatography of radioactivity appearing in venous effluent of rabbit kidney previously loaded with [^{3}H]-noradrenaline.

Sample	*No. of expts.*	*'Acid metabolites'*	*Noradrenaline*	*Normetanephrine*
Stimulated	7	16.8 ± 2.3	81.3 ± 2.2	1.3 ± 0.4
Resting	5	74.4 ± 2.3	14.4 ± 1.1	10.0 ± 1.5

Perfusate samples withdrawn before and during nerve stimulation. Chromatographic values presented as relative distribution (per cent) of recovered 'acid metabolites' (mainly deaminated products), intact noradrenaline and normetanephrine, and given as means ± s.e. mean.

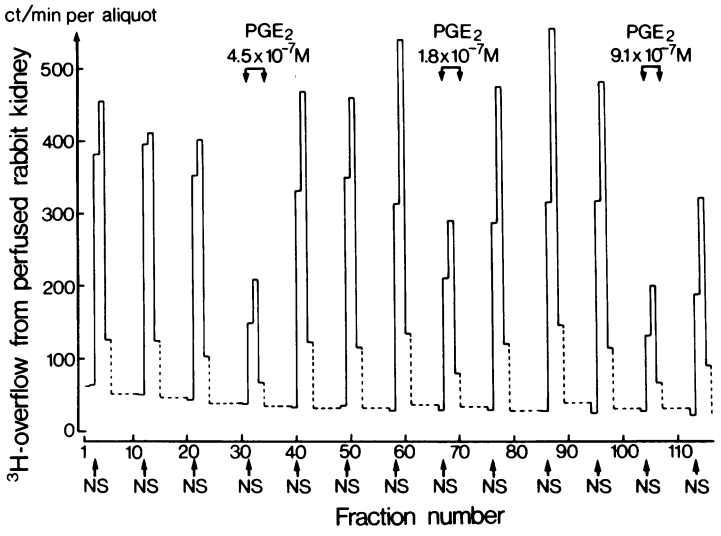

Figure 1 Isolated, perfused kidney of the rabbit, preloaded with [^{3}H]-noradrenaline. Effect of prostaglandin E_2 (PGE_2) on overflow of tracer resulting from renal nerve stimulation (NS), 150 pulses at 5 Hz. Time in min = fraction numbers. Dotted lines represent uncollected fractions.

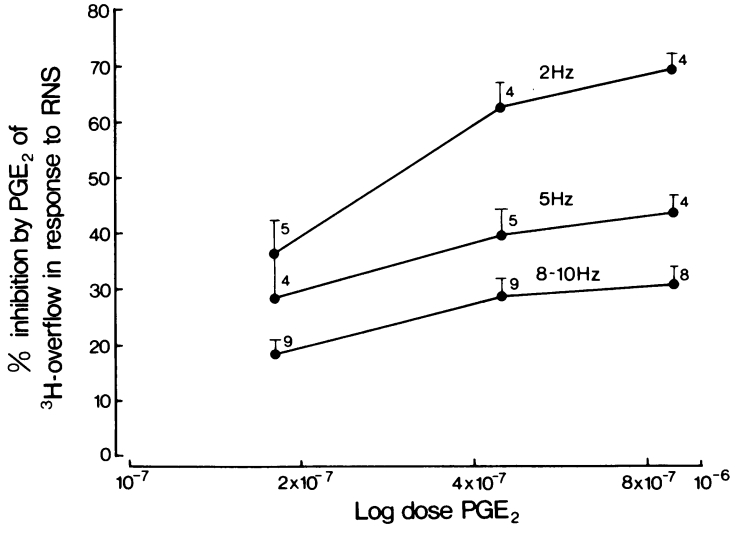

Figure 2 Inhibitory effect of prostaglandin E_2 (PGE_2) on transmitter overflow resulting from renal nerve stimulation (RNS), 150 pulses, 2-10 Hz. Vertical bars show s.e. mean. Figures at the points = number of observations.

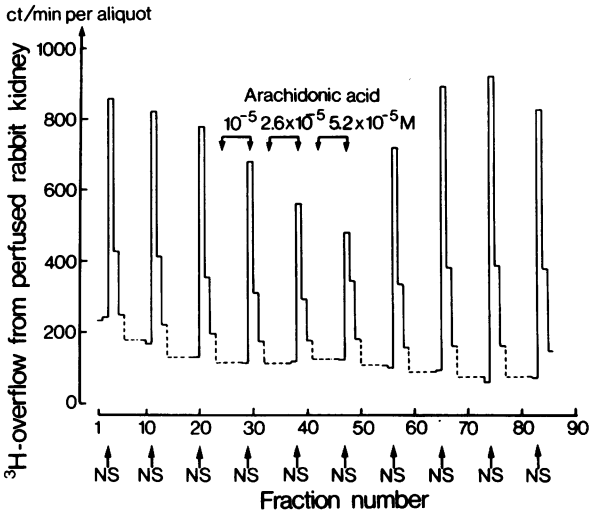

Figure 3 Effect of cumulative doses of arachidonic acid on transmitter overflow resulting from renal nerve stimulation (NS), 150 pulses at 5 Hz.

with the stimulation frequency employed (Figure 2). The vascular responses to RNS were similarly inhibited and there was partial or complete recovery from both [^{3}H]-NA overflow and vascular-inhibition 10 min after ending the infusion of prostaglandin E_2.

Effect of arachidonic acid

When arachidonic acid (2.6 x 10^{-5}M) was infused 5 min prior to and during RNS, transmitter overflow was inhibited by 35.3% (see Table 2). On the addition of cumulative doses of arachidonic acid, a dose-dependent inhibition was observed (Figure 3). Concomitantly there was a corresponding reduction of the vascular response to RNS. In most of the experiments, a slight reduction in perfusion pressure was observed throughout the duration of the infusion. Partial or complete recovery from the inhibition usually occurred within 10-20 min of ending the arachidonic acid infusion, although in some experiments the transmitter overflow remained depressed.

Effect of prostaglandin synthesis inhibitors

Indomethacin was used to block the synthesis of endogenous prostaglandins (Vane, 1971) and thus to evaluate the possible role of the latter in adrenergic transmission in the kidney.

At a stimulation frequency of 5 Hz (150 pulses), infusion of indomethacin (4.4 x 10^{-5}M) sometimes caused a slight increase in the resting perfusion pressure (up to 5 mmHg) and either no effect or a slight decrease in the effector responses to RNS. The effect on transmitter overflow, however, was much more marked. The overflow of [^{3}H]-NA during RNS increased significantly 8 min after the start of an infusion of indomethacin and plateaued after about 15 min of infusion. The maximal increase of [^{3}H]-NA overflow in response to RNS was 41.5 ± 7.0% (mean ± s.e. mean, n = 13). This value was significant at the $P < 0.001$ level according to Student's t-test for paired varieties. After ending the infusion, the transmitter overflow fell off approaching the preinfusion level within 10 to 20 min. However, in some experiments the transmitter overflow remained at an elevated level throughout post-infusion controls (Figure 4).

In three additional experiments, meclofenamic acid, (5 x 10^{-5}M) which is also a potent inhibitor of prostaglandin synthesis (Gryglewski & Vane, 1972), was used instead of indomethacin. The increase in [^{3}H]-NA overflow in response to RNS produced by infusion of meclofenamic acid was 23.3 ± 3.5% ($P < 0.05$). The drug caused qualitatively the same effects as indomethacin, although it was less potent on an equimolar basis.

Effect of prostaglandin E_2 and arachidonic acid application during indomethacin treatment

During indomethacin treatment, prostaglandin E_2 was still effective in producing an inhibition of [^{3}H]-NA overflow in response to RNS. The

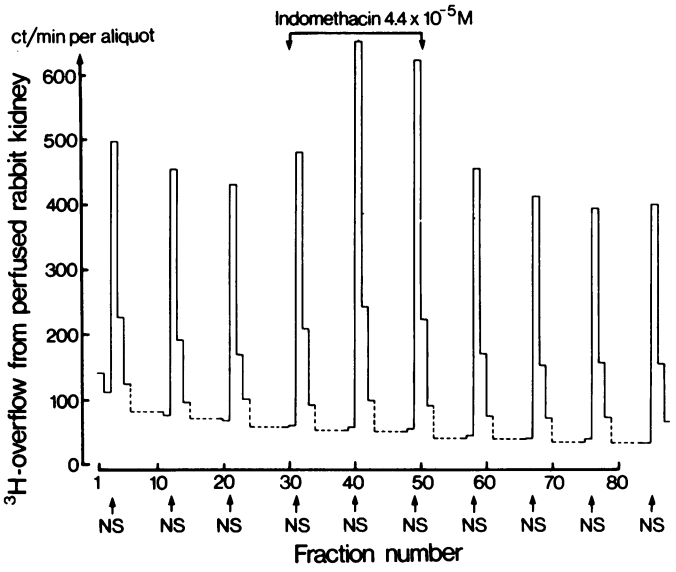

Figure 4 Effect of indomethacin on transmitter overflow resulting from renal nerve stimulation (NS), 150 pulses at 5 Hz.

inhibition produced was, in fact, significantly greater ($P < 0.02$) than that produced by prostaglandin E_2 alone, the values being 61.3% for indomethacin plus prostaglandin E_2 and 39.6% for prostaglandin E_2 alone (Table 2).

The inhibitory effect of arachidonic acid, unlike that of prostaglandin E_2, was significantly reduced ($P < 0.001$) from 35.3% for arachidonic acid alone to 10.6% for arachidonic acid in the presence of indomethacin. This would suggest that the inhibitory effect was primarily that of newly formed prostaglandin rather than that of the arachidonic acid itself (Figure 5, Table 2).

Discussion

It was previously shown that prostaglandin E_2 inhibits renal vascular responses to RNS and, to a lesser extent, exogenous NA in the dog and rabbit kidney (Lonigro *et al.*, 1973; Frame *et al.*, 1974). Those results merited the suggestion of a primary prejunctional action of prostaglandin E_2, as observed in other tissues (cf. Hedqvist, 1973).

The overflow of NA from an adrenergically innervated organ can be monitored by determining the efflux of [^{3}H]-NA previously taken up by the nerves (Hertting & Axelrod, 1961). Since in the

Table 2 Percentage change of transmitter overflow to RNS (5 Hz, 150 pulses) by prostaglandin E_2 (PGE_2) (4.5×10^{-7}M), arachidonic acid (2.6×10^{-5}M) and indomethacin (4.4×10^{-5}M), and by prostaglandin E_2 and arachidonic acid in the presence of indomethacin.

Administered compound	*Pretreatment*		*Difference*
	None	*Indomethacin*	*None − indomethacin*
PGE_2	−39.6 ± 4.5(5)	−61.3 ± 4.6(3)	21.7 ± 6.4
	$P < 0.001$	$P < 0.01$	$P < 0.02$
Arachidonic acid	−35.3 ± 4.0(5)	−10.6 ± 4.4(5)	24.7 ± 5.9
	$P < 0.001$	$P < 0.1$(NS)	$P < 0.001$
Indomethacin	+41.5 ± 7.0(13)	−	−
	$P < 0.001$		

Mean values ± s.e. mean, figures within brackets = number of experiments. Statistical analysis according to Student's *t*-test.

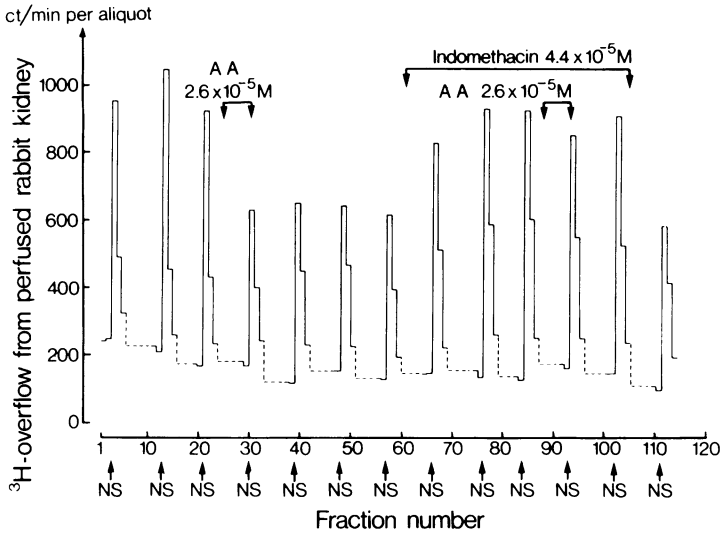

Figure 5 Effect of arachidonic acid (AA) alone and of arachidonic acid in the presence of indomethacin on transmitter overflow resulting from renal nerve stimulation (NS, 150 pulses at 5 Hz).

present study the majority of the radioactivity overflowing in response to RNS was represented by intact [^{3}H]-NA, total radioactivity was considered an appropriate indicator of endogenous NA overflow.

It is apparent from this study that transmitter overflow resulting from RNS is dose-dependently and reversibly inhibited by exogenous prostaglandin E_2 and can also be modified by altering endogenous prostaglandin levels. Judging from experiments on the cat spleen and the guinea-pig vas deferens and heart (Hedqvist, 1970, 1974a; Bhagat, Dhalla, Ginn, LaMontagne & Montier, 1972), where prostaglandin E_2 does not alter the disposition of released NA, it is likely that the observed inhibition of transmitter overflow represents an action directly on the process of excitation-secretion coupling in the nerve terminals.

In examining the inhibitory or 'braking' effect of exogenous prostaglandin E_2 on transmitter release, the control transmitter overflow to RNS acted as a guide to the effect of prostaglandin E_2. However, it could not be regarded as a true control since, at least in the dog and rabbit kidney, RNS causes release of prostaglandin E_2 which should also provide some braking action (Dunham & Zimmermann, 1970; Davis & Horton, 1972; McGiff, Crowshaw, Terragno, Malik & Lonigro, 1972). In an attempt to reduce the interference of endogenous prostaglandins, experiments were carried out in which prostaglandin synthesis inhibitors were administered to the kidney. The primary effect of the synthesis inhibitors, indomethacin and meclofenamic acid (Vane, 1971; 1973), was to elevate transmitter overflow, which is to be expected if an endogenous braking-mechanism has been partially or wholly removed. Moreover, in harmony with previous observations on the guinea-pig vas deferens (Hedqvist, 1974a), a given dose of prostaglandin E_2 showed an increased ability to inhibit transmitter overflow during indomethacin infusion. This increased potency may also be attributable to removal of a local braking mechanism, assuming that administered prostaglandin E_2 normally has to compete with endogenous prostaglandin for active sites in the nerve terminals.

In a series of experiments the inhibitory effect of prostaglandin E_2 on transmitter release was studied at different stimulation frequencies. Generally the inhibition produced by a given dose varied inversely with the stimulation frequency employed. This would seemingly suggest an endogenous prostaglandin-mediated braking mechanism which operates more readily at a low impulse activity than at a high. This is in good agreement with results obtained in the rabbit heart (Junstad & Wennmalm, 1973) where the sympathetic transmitter-release-mechanism became less sensitive to exogenous prostaglandin E_2 as the frequency of stimulation was increased from 2-10 Hz. The release of *endogenous* prostaglandin during sympathetic nerve stimulation of the dog

kidney and the rabbit heart shows no apparent linear correlation with either the frequency or the transmitter overflow (Dunham & Zimmerman, 1970; Junstad & Wennmalm, 1973). Thus an increase in stimulation frequency from 2-10 Hz only approximately doubled the efflux of prostaglandin E_2-like material per unit time whilst the transmitter overflow increased tenfold. It would seem, therefore, that the endogenous transmitter braking-mechanism is capable of compensating for changes in impulse activity but that its effectiveness is limited both by the amount of prostaglandin releasable and by the sensitivity of the NA-release process to prostaglandin.

The series of experiments in which the prostaglandin precursor arachidonic acid was used, was carried out in an attempt to show that newly synthesized prostaglandins have access to sympathetic junctions. Infusion of arachidonic acid produced a marked inhibitory effect on both transmitter overflow and vascular responses to RNS. This effect was mainly due to the formation of prostaglandins since the effect was very much reduced or abolished when arachidonic acid was infused in the presence of indomethacin. A small residual effect of the arachidonic acid is not unexpected, since indomethacin in doses similar to that used in the present study has been shown to inhibit markedly but not abolish the conversion of arachidonic acid to prostaglandin E_2 in rabbit kidney and in rat kidney homogenates (Larsson & Änggård, 1974; Fredholm, Hedqvist & Larsson, unpublished observations).

In discussing a role for prostaglandins as regulators of transmitter release at renal sympathetic nerve endings, the assumption is made that there is a ready source of prostaglandins in the vicinity of the cortical nerve endings. The biosynthesis of prostaglandins, however, has been shown to be ten times greater in the medulla than in the cortex (Larsson & Änggård, 1973). It has, nevertheless, been emphasized by these authors that cortical synthesis is large enough to be of significance. The renal cortex, in addition to its biosynthetic capacity, possesses an abundant amount of prostaglandin degrading enzyme, prostaglandin dehydrogenase (Änggård, Larsson & Samuelsson, 1971). It is probable, however, that endogenous cortical prostaglandin which escapes degradation may have access to the cortical nerve endings. This possibility is inhanced by the fact that prostaglandin E_2 infused into the present system, although going directly to the cortex, produced an effect on RNS. An alternative suggestion is that medullary prostaglandins may reach the juxtamedullary arterioles via the vasa recta (Larsson & Änggård, 1973).

Although both the juxtamedullary and outer cortical arterioles have adrenergic innervation, it is interesting that during RNS or induction of stress in rabbits, the outer cortex becomes ischaemic whilst juxtamedullary cortical blood flow persists (Trueta, Barclay, Daniel, Franklin & Pritchard, 1947). Moreover, the prostaglandin synthesis capacity of the cortex increases towards the medulla and stimulation of prostaglandin synthesis using arachidonic acid increases juxtamedullary blood flow in the rabbit (Larsson & Änggård, 1973; 1974). It seems, therefore, that prostaglandins are primarily available in the juxtamedullary zone to inhibit sympathetic arteriolar tone. It is apparent from the present study that this dilatation or prevention of vasoconstriction is, at least in part, attributable to inhibition of transmitter release, i.e. a prejunctional action on adrenergic neuro-effector transmission. However, part of the effect may be post-junctionally located at the effector cell level since prostaglandin E_2 to some extent inhibits vascular responses to NA in the dog and rabbit kidney (McGiff *et al.* 1972; Frame *et al.*, 1974) and newly formed prostaglandin increases juxtamedullary blood flow in the dog kidney under *in vitro* conditions (Itskovitz, Terragno & McGiff, 1974).

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References

ÄNGGÅRD, E., LARSSON, C. & SAMUELSSON, B. (1971). The distribution of 15-hydroxyprostaglandin dehydrogenase and prostaglandin-Δ^{13}-reductase in tissues of the swine. *Acta physiol. scand.*, **81**, 396-404.

BHAGAT, B., DHALLA, N.S., GINN, D., LA MONTAGNE, A.E. & MONTIER, A.D. (1972). Modification by prostaglandin E_2 (PGE_2) of the response of guinea-pig isolated vasa deferentia and atria to adrenergic stimuli. *Br. J. Pharmac.*, **44**, 689-698.

CROWSHAW, K. (1971). Prostaglandin biosynthesis from endogenous precursors in rabbit kidney. *Nature, New Biol.*, **231**, 240-242.

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.

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

FRAME, M.H. & HEDQVIST, P. (1974). Effects of prostaglandin E_2 on the sympathetic neuroeffector system of the rabbit kidney in vitro and in situ. *Acta physiol. scand.*, **91**, 25A.

FRAME, M.H., HEDQVIST, P. & ÅSTRÖM, A. (1974). Effects of prostaglandin E_2 on vascular responses of the rabbit kidney to nerve stimulation and noradrenaline, in vitro and in situ. *Life Sci.*, **15**, 239-244.

FREDHOLM, B. & HEDQVIST, P. (1973). Role of pre- and postjunctional inhibition by prostaglandin E_2 of lipolysis induced by sympathetic nerve stimulation in dog subcutaneous adipose tissue in situ. *Br. J. Pharmac.*, **47**, 711-718.

GRYGLEWSKI, R. & VANE, J.R. (1972). The release of prostaglandins and rabbit aorta contracting substance (RCS) from rabbit spleen and its antagonism by anti-inflammatory drugs. *Br. J. Pharmac.*, **45**, 37-47.

HAMBERG, M. (1969). Biosynthesis of prostaglandins in the renal medulla of rabbit. *FEBS Lett.*, **5**, 127-130.

HEDQVIST, P. (1970). Studies on the effect of prostaglandins E_1 and E_2 on the sympathetic neuromuscular transmission in some animal tissues. *Acta physiol. scand.*, **79**, Suppl. 345, 1-40.

HEDQVIST, P. (1973). Prostaglandin mediated control of sympathetic neuroeffector transmission. *Adv. Biosci.*, **9**, 461-473.

HEDQVIST, P. (1974a). Prostaglandin action on noradrenaline release and mechanical responses in the stimulated guinea-pig vas deferens. *Acta physiol. scand.*, **90**, 86-93.

HEDQVIST, P. (1974b). Aspects of prostaglandin and α-receptor mediated control of transmitter release from adrenergic nerves. In *Frontiers in Catecholamine Research*, ed. Usdin, E. & Snyder, S., pp. 583-587. Oxford: Pergamon Press.

HERBACZYNSKA-CEDRO, K. & VANE, J.R. (1973). Contribution of intrarenal generation of prostaglandin to autoregulation of renal blood flow in the dog. *Circulat. Res.*, **33**, 428-436.

HERBACZYNSKA-CEDRO, K. & VANE, J.R. (1974). Prostaglandins as mediators of reactive hyperaemia in kidney. *Nature, Lond.*, **247**, 492.

HERTTING, G. & AXELROD, J. (1961). Fate of tritiated noradrenaline at the sympathetic nerve endings. *Nature, Lond.*, **192**, 172-173.

ITSKOVITZ, H.D., TERRAGNO, N.A. & McGIFF, J.C. (1974). Effect of a renal prostaglandin on distribution of blood flow in the isolated canine kidney. *Circulat. Res.*, **34**, 770-776.

JUNSTAD, M. & WENNMALM, Å. (1973). Prostaglandin mediated inhibition of noradrenaline release at different impulse frequencies. *Acta physiol scand.*, **89**, 544-549.

LARSSON, C. & ÄNGGÅRD, E. (1973). Regional differences in the formation and metabolism of prostaglandins in the rabbit kidney. *Eur. J. Pharmac.*, **21**, 30-36.

LARSSON, C. & ÄNGGÅRD, E. (1974). Increased juxtamedullary blood flow on stimulation of intrarenal prostaglandin biosynthesis. *Eur. J. Pharmac.*, **25**, 326-334.

LEE, J.B., CROWSHAW, K., TAKMAN, B.H., ATTREP, K. & GOUGOUTAS, J.Z. (1967). The identification of prostaglandins E_2, $F_{2\alpha}$ and A_2 from rabbit kidney medulla. *Biochem. J.*, **105**, 1251-1260.

LONIGRO, A.J., TERRAGNO, N.A., MALIK, K.U. & McGIFF, J.C. (1973). Differential inhibition by prostaglandins of the renal actions of pressor stimuli. *Prostaglandins*, **3**, 595-606.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A. & LONIGRO, A.J. (1970). Renal prostaglandins: Possible regulators of the renal actions of pressor hormones. *Nature, Lond.*, **227**, 1255-1257.

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. Sci.*, **42**, 223-233.

McGIFF, J.C. & ITSKOVITZ, H.D. (1973). Prostaglandins and the kidney. *Circulat. Res.*, **33**, 479-488.

NEEDLEMAN, P., DOUGLAS, Jr., J.R., JALSETIK, B., STOECKLEIN, P.B. & JOHNSON, Jr., E.M. (1974). Release of renal prostaglandin by catecholamines: Relationship to renal endocrine function. *J. Pharmac. exp. Ther.*, **188**, 453-460.

TRUETA, J., BARCLAY, A.E., DANIEL, P.M., FRANKLIN, K.J. & PRITCHARD, M.M.L. (1947). *Studies of the renal circulation.* Oxford: Blackwell Scientific Publ.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.*, **231**, 232-235.

VANE, J.R. (1973). Inhibition of prostaglandin biosynthesis as the mechanism of action of aspirin-like drugs. *Adv. Biosci.*, **9**, 395-411.

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