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EFFECTS OF NERVE STIMULATION AND OF ADMINISTRATION OF NORADRENALINE OR POTASSIUM CHLORIDE UPON THE RELEASE OF PROSTAGLANDINS I_2, E_2 AND $F_{2\alpha}$ FROM THE PERFUSED MESENTERIC ARTERIAL BED OF THE RABBIT

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1 The release of prostaglandin I_2 (PGI_2), PGE_2 and $PGF_{2\alpha}$ from the perfused mesenteric arterial bed of the rabbit was examined at rest, following nerve stimulation and following noradrenaline (NA) or potassium chloride (KCl) administration.

2 Stimulation of adrenergic nerves at 10 Hz caused a significant increase in the release of both PGI_2, (assayed in terms of 6-oxo-$PGF_{1\alpha}$) and PGE_2 but a significant decrease in the release of $PGF_{2\alpha}$.

3 Exogenous Na (2 μg) increased the output of PGI_2 and PGE_2 but left the output of $PGF_{2\alpha}$ unaffected.

4 KCl (15 mg) significantly increased the output of $PGF_{2\alpha}$ but left the output of PGI_2 unaffected.

5 It is concluded that PGI_2 and PGE_2 output from the mesenteric arterial bed of the rabbit increases following stimulation of adrenoceptors. The sympathetic nervous system may therefore modulate PGI_2/platelet interaction.

6 Prostaglandins released from the blood vessels by sympathetic nerve stimulation may also modulate adrenergic transmission to the blood vessels.

Introduction

Prostaglandins of the E series may exert a modulating influence at the adrenergic neuroeffector junction by both controlling the release of noradrenaline (NA) from the nerve endings and by exerting an effect at the postsynaptic site (Hedqvist, 1977; Westfall, 1977). Prostaglandins of the E series are released following adrenergic nerve stimulation, from many but not all tissues, and when released may play a role in the feedback regulation of NA output. The effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on release of NA is less clear (Hedqvist, 1977).

PGI_2, the main prostaglandin produced by the blood vessels, (Moncada & Vane, 1978) reduces vasoconstrictor responses to nerve stimulation and to exogenous catecholamines in some tissues (Lippton, Chapnick, Hyman & Kadowitz, 1979; Hedqvist, 1979; Yabek & Avner, 1980). However, it does not appear to affect transmitter release from the adrenergic nerves (Armstrong & Thirsk, 1979; Hedqvist, 1979).

Although the production of PGI_2 by the blood vessels has been studied extensively, its release following nerve stimulation and following the stimulation of adrenoceptors or non-adrenergic stimuli has not been examined. In the present study, the release of PGI_2, PGE_2 and $PGF_{2\alpha}$ from the perfused mesenteric arterial bed of the rabbit, *in vitro*, has been measured following nerve stimulation and following exogenous NA or potassium chloride (KCl) administration.

Methods

The isolated perfused mesenteric bed of the rabbit

Male albino rabbits weighing 2 to 3 kg were anaesthetized with sodium pentobarbitone (30 mg/kg intravenously). The abdomen was opened and the superior mesenteric artery was cannulated. The artery was then excised along with its small resistance vessels as described by McGregor (1965). The isolated vessels were flushed with saline and transferred to a thermostatically controlled box (maintained at 37°C). The mesenteric vessels were perfused with McEwen's solution of the following composition (g/l): NaCl 7.6, $NaHCO_3$ 2.1, KCl 0.42, $CaCl_2$ 0.24, NaH_2PO_4 0.143, glucose 2.0 and sucrose 4.5. This solution was bubbled with 5% CO_2, 95% O_2 and perfused through the vessels at a constant rate of 5 ml/min by means of a peristaltic pump (Heidolph Elektro K G). The per-

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fusion pressure was recorded via a side arm with a pressure transducer (Statham) connected to a Grass polygraph (7C). A platinum electrode was placed around the periarterial nerve plexus and the nerves were stimulated at 10 Hz, using supramaximal biphasic rectangular pulses of 1 ms duration, for 15 s at 30 min intervals. NA (2 μg) and KCl (15 mg) were given as single injections in 0.1 ml into the arterial cannula at 30 min intervals. Each arterial bed was exposed to only one type of stimulus (nerve stimulation or the administration of NA or KCl). This stimulus was repeated three times within each experiment and four experiments were performed for each type of stimulus. All preparations were perfused for an equilibration period of 1 h before any treatment was started. Perfusion fluid leaving the arterial bed was led from the thermostatically controlled box by a piece of polyethelene tubing to a fraction collector. The dead space between the preparation and the fraction collector was 0.5 ml. Samples of perfusate were collected for 1 min every 10 min at rest, and every 1 min from 2 min before to 6 min after stimulation of the nerves or the administration of NA or KCl. Each sample was assayed for PGI_2 (as 6-oxo-$PGF_{1\alpha}$), PGE_2 and $PGF_{2\alpha}$.

Measurement of prostaglandins

Prostaglandins present in the perfusate were measured by radioimmunoassay (RIA). $PGF_{2\alpha}$ was assayed using an antibody raised in rabbits and tested in this laboratory (Dighe, Emslie, Henderson, Rutherford & Simon, 1975). The antibody has low cross reactivities (<4%) with other prostaglandins (Poyser & Scott, 1980).

PGE_2 was assayed using an antibody raised in rabbits and purchased from the Pasteur Institute (Paris). This antibody has low cross-reactivities with PGE_1 (6.6%) and the other prostaglandins (<0.5%, Poyser & Scott, 1980).

PGI_2 was measured in terms of its stable hydrated product, 6-oxo-$PGF_{1\alpha}$, using an antibody raised in rabbits and tested in this laboratory. It has low cross-reactivities with $PGF_{2\alpha}$ (5.8%), PGE_2 (6.8%), PGE_1 (2.0%) and other prostaglandins (<0.1%, Poyser & Scott, 1980). The sensitivities of the assays, for $PGF_{2\alpha}$, PGE_2 and 6-oxo-$PGF_{1\alpha}$ were 25 pg, 4 pg and 25 pg respectively.

Initially samples of perfusate were acidified to pH 4.0 with 1 M HCl and extracted 3 times with 2 volumes of ethyl acetate. The ethyl acetate fractions were combined, evaporated to dryness and the prostaglandin content of the extract measured. However, it was found later that samples of perfusate assayed directly or after extraction gave identical results so the extraction step was omitted. Each sample was assayed in triplicate for $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ and in duplicate for PGE_2. The prostaglandin output was expressed as ng/min.

The intra-assay coefficients of variation were calculated from the variation between the triplicate or duplicate values obtained for each sample and were found to be 14.3%, 9.3% and 10.1% for $PGF_{2\alpha}$, PGE_2 and 6-oxo-$PGF_{1\alpha}$ respectively. A known quantity (160 pg $PGF_{2\alpha}$, 20 pg PGE_2 and 160 pg 6-oxo-$PGF_{1\alpha}$) was incorporated into each of their respective assays to indicate the accuracy of measurement. Mean (±s.e. mean, $n = 6$) results obtained were: 160.2 ± 14.1 pg $PGF_{2\alpha}$, 19.7 ± 1.2 pg PGE_2 and 178.3 ± 14.1 pg 6-oxo-$PGF_{1\alpha}$.

Statistics

Results were compared by a paired t test. In all experiments, statistical significance was taken to be $P < 0.05$.

Drugs

Noradrenaline was dissolved in distilled water and ascorbic acid was added to prevent oxidation. The final dilution was made up in McEwen's solution. Potassium chloride was dissolved in distilled water. The sources of the drugs were: (−)-noradrenaline bitartrate (Sigma, Poole), potassium chloride (analar grade, BDH Chemicals Ltd., Poole).

Results

The effect of nerve stimulation on the release of prostaglandins I_2, E_2 and $F_{2\alpha}$

The major prostaglandin released into the superfusate of the mesenteric arterial bed was PGI_2 (expressed in terms of 6-oxo-$PGF_{1\alpha}$) with lesser amounts of $PGF_{2\alpha}$ and PGE_2.

Nerve stimulation caused an increase in the output of PGI_2 and PGE_2 and a reduction in the output of $PGF_{2\alpha}$. Figure 1 shows the time course of the release of PGI_2 (as 6-oxo-$PGF_{1\alpha}$), PGE_2 and $PGF_{2\alpha}$ at rest and following nerve stimulation in a typical experiment.

Regarding all the experiments the output (mean ± s.e. mean, n = 12) of 6-oxo-$PGF_{1\alpha}$ significantly increased ($P < 0.001$) from 9.64 ± 0.87 ng/min at 1 min before stimulation to 17.8 ± 1.8 ng/min within 2 min after stimulation. There was also a significant increase ($P < 0.02$) in the output ($n = 12$) of PGE_2 from 1.53 ± 0.36 ng/min at 1 min before stimulation to 3.08 ± 0.76 ng/min within 2 min after stimulation. The output ($n = 12$) of $PGF_{2\alpha}$ was significantly ($P < 0.001$) reduced from 2.69 ± 0.44 ng/min at 1 min

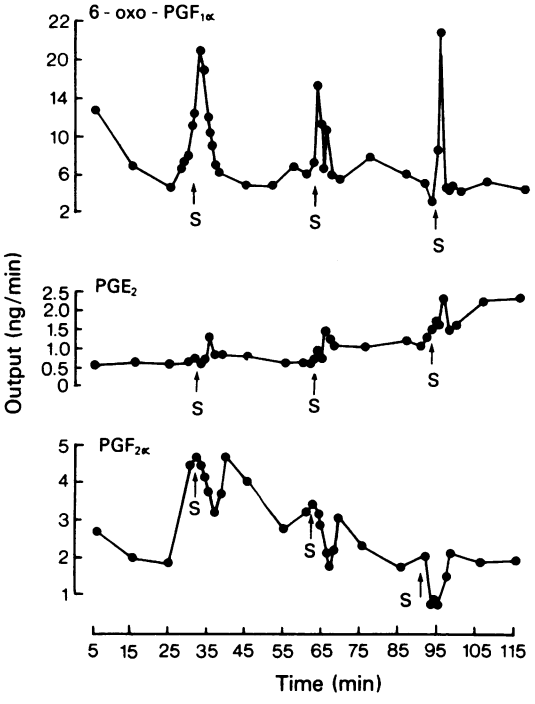

Figure 1 Effect of nerve stimulation (10 Hz) on the release of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), prostaglandin E_2(PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the perfused mesenteric arterial bed of the rabbit in a typical experiment: (●) indicates prostaglandin output at specific times of collection of samples of perfusate. S indicates time of nerve stimulation.

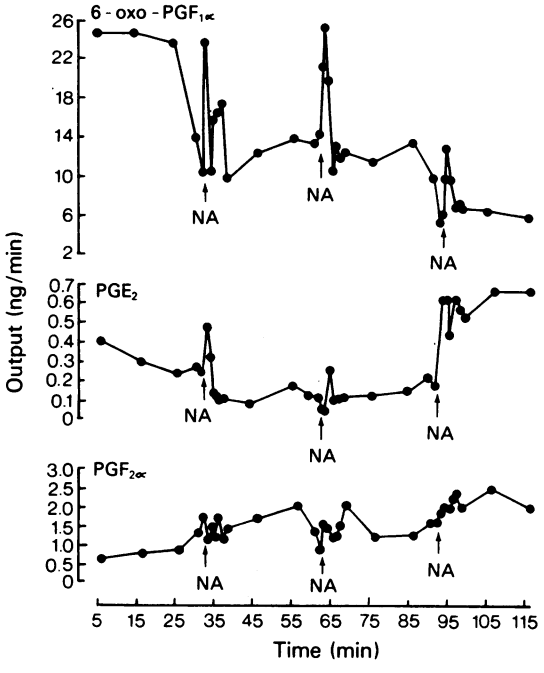

Figure 2 Effect of administration of noradrenaline (2 μg) on the release of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the perfused mesenteric arterial bed of the rabbit as shown in a typical experiment: (●) indicates prostaglandin output at specific times of collection of samples of perfusate. NA indicates time of administration of noradrenaline.

before stimulation to 1.42 ± 0.25 ng/min within 2 min after stimulation.

The output of PGI_2, PGE_2 and $PGF_{2\alpha}$ returned to near normal basal levels within 5 min following stimulation, though in some experiments the basal output of 6-oxo-$PGF_{1\alpha}$ tended to decrease while the output of PGE_2 tended to increase with time during the total perfusion period. The basal output of $PGF_{2\alpha}$ remained fairly stable.

The effect of noradrenaline (2 μg) on the release of prostaglandins I_2, E_2 and $F_{2\alpha}$

Addition of NA increased the output of PGI_2 and PGE_2 with a similar time course to that observed following nerve stimulation. The output of $PGF_{2\alpha}$ remained unchanged.

Figure 2, shows the time course of the release of the three prostaglandins at rest and following NA administration in a typical experiment.

Regarding all experiments the output ($n = 12$) of both 6-oxo-$PGF_{1\alpha}$ and PGE_2 significantly increased ($P < 0.001$ and $P < 0.05$ respectively) from 5.0 ± 1.2 ng/min and 0.29 ± 0.11 ng/min at 1 min before the administration of NA to 10.3 ± 2.2 ng/min and 0.51 ± 0.16 ng/min within 2 min after the administration of NA, respectively. The output of $PGF_{2\alpha}$ ($n = 12$) 1 min before and 2 min after the administration of NA was 0.64 ± 0.18 and 1.01 ± 0.32 respectively. These values were not significantly different when compared by the paired t test.

The effect of KCl (15 mg) on the release of prostaglandins I_2, E_2 and $F_{2\alpha}$

Administration of KCl (15 mg) did not affect the release of PGI_2 (expressed as 6-oxo-$PGF_{1\alpha}$) or PGE_2 but it caused an increase in the output of $PGF_{2\alpha}$. Figure 3 shows a typical experiment. In all experiments ($n = 12$) the output of $PGF_{2\alpha}$ increased significantly ($P < 0.01$) from 1.65 ± 0.22 ng/min at 1 min before to 2.59 ± 0.42 ng/min within 2 min after the addition of KCl. The outputs, ($n = 12$) of 6-oxo-$PGF_{1\alpha}$ and PGE_2 were 19.4 ± 2.7 ng/min and

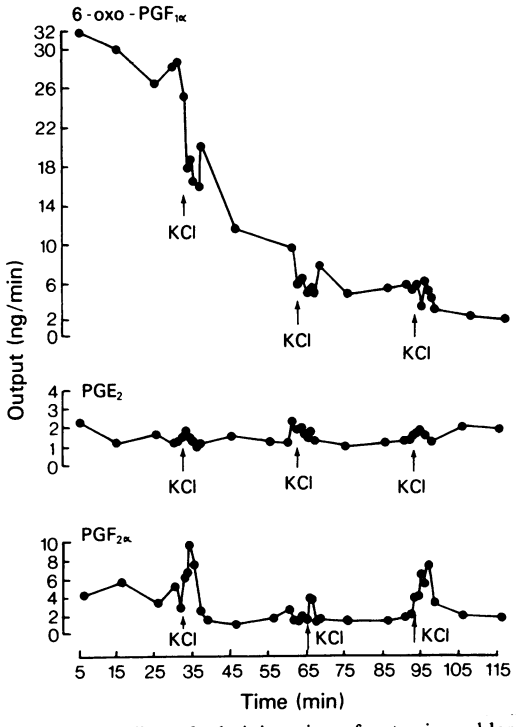

Figure 3 Effect of administration of potassium chloride (15 mg) on the release of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the perfused mesenteric arterial bed of the rabbit as shown in a typical experiment: (●) indicates prostaglandin output at specific times of collection of samples of perfusate. KCl indicates time of administration of potassium chloride.

1.07 ± 0.32 ng/min respectively 1 min before the administration of KCl and were 22.6 ± 3.1 ng/min and 1.13 ± 0.21 ng/min respectively within 2 min after this administration.

Discussion

The perfused mesenteric artery of the rabbit released PGI_2, PGE_2 and $PGF_{2\alpha}$ into the perfusing medium with PGI_2 being the major prostaglandin released. Sympathetic nerve stimulation or administration of NA caused a rapid increase in both the output of PGI_2 and PGE_2. The release of $PGF_{2\alpha}$ was inhibited by nerve stimulation whereas exogenous NA had no effect. The administration of KCl in a dose that produced comparable vasoconstriction to nerve stimulation or exogenous NA did not affect the output of PGI_2 and PGE_2 but, rather surprisingly, did increase the release of $PGF_{2\alpha}$. It is clear from these comparisons that the effects of nerve stimulation and exogenous NA are due to stimulation of adrenoceptors and not due to vasoconstriction *per se*.

There is evidence that PGI_2 produced by the blood vessels may prevent platelet aggregation (Moncada & Vane, 1978). Our study has indicated that sympathetic nerve stimulation and circulating catecholamines may stimulate the output of PGI_2 from blood vessels and may be important physiologically in controlling PGI_2/platelet interactions.

Prostaglandins released from blood vessels may also have important actions on the blood vessels directly and/or their sympathetic nerves' supply. PGE_2 and PGI_2 are both vasodilators and may be released on nerve stimulation to aid in the reduction of tone of the blood vessels following vasoconstriction produced by NA. $PGF_{2\alpha}$ is usually considered to constrict blood vessels, a decrease in its output following nerve stimulation may also aid in the reduction of tone. However, it is difficult to explain at present why nerve stimulation but not addition of NA reduces the output of $PGF_{2\alpha}$. Perhaps differences in accessibility between endogenous and exogenous NA to the sites that produce $F_{2\alpha}$ may be responsible, or the release of $PGF_{2\alpha}$ (or lack of it) may be connected with ionic changes occurring in the nerve when stimulated. It is also difficult to explain why the output of $PGF_{2\alpha}$ should increase after the administration of KCl.

There is much evidence that PGE_2 is released from many but not all tissues following sympathetic nerve stimulation and that it can act back on the sympathetic nerve ending to reduce the release of NA (Hedqvist, 1977). PGE_2 does reduce the output of PGE_2 from the mesenteric arteries of the rabbit (Armstrong & Thirsk, 1979) so the PGE_2 released in our experiments may act on the nerve to decrease the output of NA. However, we have yet to investigate this suggestion. PGI_2 is reported not to affect release of NA from sympathetic nerves in the perfused kidney of the rabbit (Hedqvist, 1979) and from the mesenteric arteries of the rabbit (Armstrong & Thirsk, 1979). It is unlikely therefore that PGI_2 influences the release of transmitter from nerve endings in blood vessels. Studies indicate that $PGF_{2\alpha}$ does not inhibit release of NA from nerve endings (Hedqvist & Wennmalm, 1971; Hedqvist, 1976; Frame, 1976) and may in fact facilitate release in several tissues (Hedqvist, 1977). The role of $PGF_{2\alpha}$, if any, in the release of NA from sympathetic nerves to blood vessels is far from clear.

As well as modulating release of adrenergic transmission presynaptically, there is evidence that prostaglandins may also modulate transmission postsynaptically. PGE_2 reduces the effect of exogenous NA on the rabbit mesenteric arteries (Malik & McGiff, 1974) as well as in other vascular tissues (Hedqvist, 1977). PGI_2 also reduces the responses to exogenous NA in

the vascular beds of some tissues (Lippton *et al.*, 1979; Hedqvist, 1979; Yabeh & Avner, 1980). $PGF_{2\alpha}$ on the other hand enhances vasoconstrictor responses to exogenous NA in the pulmonary lobar artery and vein (Kadowitz, George, Joiner & Hyman, 1973) and in the canine hindlimb superficial veins (Kadowitz, Sweet & Brody, 1971).

One or more of the prostaglandins produced by blood vessels may therefore act to modulate adrenergic transmission by reducing the release of transmitter and by reducing the activity of the released NA. They may also aid in reducing blood vessel tone after constriction produced by NA by a direct action on the blood vessel. However, whether these effects of prostaglandins are of physiological significance and are confined to one or two vascular beds or are more widespread throughout the body, merits further study. Nevertheless this study indicates that the sympathetic nervous system may control the output of PGI_2 and the output of other prostaglandins from the blood vessels.

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