J. Pharm. Pharmacol. 1985, 37: 188–192
Received July 17, 1984
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Prostanoids and adrenaline release: a study of [^{3}H]adrenaline efflux from the rabbit isolated, perfused, adrenal gland

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[^{3}H]Adrenaline was incorporated in an isolated perfused preparation of the rabbit adrenal gland and the effects of indomethacin, PGE_2 and PGI_2 on its release were investigated. Efflux of [^{3}H]adrenaline was elicited by electrical stimulation of the splanchnic nerve (60 s at 5 Hz). Indomethacin (3 and 30 μM) had no effect on stimulation-induced efflux. PGE_2 (30, 90 and 300 nM) reduced the efflux; with 90 nM PGE_2 the inhibition amounted to approximately 30%. PGI_2, in concentrations from 90 to 600 nM, was without effect. These findings indicate that release of [^{3}H]adrenaline from the rabbit adrenal gland is not subject to modulation by endogenous adrenal prostaglandins; however, PGE_2 may play a role in some pathological situations.

Prostaglandins, notably PGE_1 and PGE_2, are known to inhibit the exocytotic release of noradrenaline from sympathetic nerve endings (Stjarne 1979). Whether prostacyclin (PGI_2) has a similar effect is debatable. Khan & Malik (1982) reported that noradrenaline outflow from the sympathetic nerve endings of the rat heart is decreased by both PGE_2 and PGI_2, although in many other preparations PGI_2 does not inhibit release (Armstrong & Thirsk 1979; Hedqvist 1979; Smith et al 1982). It has been postulated that endogenously released prostaglandins have a modulatory role in adrenergic transmission, for indomethacin has been observed to increase catecholamine outflow from sympathetic nerves (Khan & Malik 1982; Smith et al 1982; Malik & McGiff 1975); however, in other studies, indomethacin failed to facilitate noradrenaline release and this hypothesis is therefore open to question (Dubocovich & Langer 1975; Armstrong & Thirsk 1979; Micalizz & Pals 1980; Imaizumi et al 1983).

Secretion of catecholamines induced by acetylcholine and potassium, from slices of bovine and rat adrenal gland, is reduced by PGE_2 (Boonyaviroj et al 1977; Boonyaviroj & Gutman 1979). In contrast, in the dog adrenal, high concentrations of PGE_2 have been reported to act synergistically with acetylcholine in evoking catecholamine secretion (Yamashita et al 1978). We have previously pointed out the discrepancies in relation to the effects of α-adrenoceptor agonists and antagonists on adrenal catecholamine release, the effects depending not only on the species, but the type of preparation and method of eliciting release of catecholamines (Collett & Story 1982). In the present study we have investigated the effects of indomethacin, PGE_2 and PGI_2 on the release of [^{3}H]adrenaline from an isolated perfused preparation of the rabbit adrenal gland evoked by electrical stimulation of the splanchnic nerve.

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MATERIALS AND METHODS

Rabbit isolated perfused adrenal gland

The dissection and preparation of the rabbit adrenal gland have been described by Collett & Story (1982). The gland was removed, the splanchnic nerve drawn through a pair of platinum ring electrodes and the preparation perfused with Krebs-Henseleit solution equilibrated with 95% oxygen and 5% carbon dioxide at 37 °C; the flow rate was kept constant at 0·5 ml min^{-1}. The catecholamine stores were radiolabelled by perfusing the gland for 20 min with Krebs-Henseleit solution containing [^{3}H]adrenaline (0·2 μM, 74 KBq ml^{-1}) followed by a 60 min washout with adrenaline-free Krebs-Henseleit solution. Efflux of radioactivity from the preparation was elicited by three 1 min periods of splanchnic nerve stimulation at 30 min intervals. The pulses were monophasic square waves of 1 ms duration and delivered at 5 Hz. The potential across the electrodes was 40 V. The efflux of radioactivity before, during and after each period of splanchnic nerve stimulation was measured in 2 min fractions of the perfusate and the content of radioactivity of each fraction was

estimated by liquid scintillation counting. The resting efflux was taken as the mean content of radioactivity of two fractions collected immediately prior to splanchnic nerve stimulation. The splanchnic nerve stimulation-induced efflux was obtained by subtracting the resting efflux from the radioactivity content of each of six subsequent fractions and summing the differences. The resting and splanchnic nerve stimulation-induced effluxes of radioactivity for the second and third periods were expressed as percentages of the corresponding values for the first period.

In experiments to examine the effects of indomethacin, the cyclo-oxygenase inhibitor was added (at 3 and 30 μM) to the Krebs-Henseleit solution 20 min before the second period of nerve stimulation and removed following collection of the last fraction associated with the second period of nerve stimulation. The resting and stimulation-induced effluxes of radioactivity were compared with those obtained in control experiments in the absence of indomethacin. To establish that indomethacin (3 or 30 μM) inhibited cyclo-oxygenase, samples of perfusion fluid were taken from non-radiolabelled preparations, before and 20 min after addition of the inhibitor. 6-Oxo-$PGF_{1\alpha}$ was measured by radioimmunoassay as described by Dusting et al (1981).

In a second series of experiments to examine the effects of exogenous prostanoids, indomethacin (3 μM) was added to the Krebs-Henseleit solution 30 min before the first period of nerve stimulation and remained throughout the experiments to suppress biosynthesis of endogenous prostanoids. PGE_2 or PGI_2 were then added 20 min before the second period of nerve stimulation and removed before the third period, and results were compared with those obtained in the presence of indomethacin alone.

Counting of radioactivity

For determination of the amounts of radioactivity in fractions of adrenal perfusate, approximately 0·2 ml of 6 M hydrochloric acid was added to each vial containing the 2 min (1 ml) fraction and then 10 ml of scintillation solution was added. The liquid scintillation solution was of the following composition: 5·5 g of 2,5-diphenyloxazole; 0·1 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene and 333 ml of Triton X made up to 1 litre in toluene. Radioactivity was measured in a Packard (model 460 CD) liquid scintillation spectrometer. The radioactivity present in each vial was expresed as Bq (= disintegration s^{-1}), corrections being made for counting efficiency by automatic external standardization.

Physiological salt solutions

Krebs-Henseleit solution was of the following composition (mM): NaCl, 118; KCl, 4·7; Na_2HCO_3, 25; KH_2PO_4, 1·03; $MgSO_4.2\ H_2O$, 0·45; $CaCl_2$, 2·5; D-(+)-glucose, 11·1. Ethylenediaminetetraacetic acid (0·067 mM) and ascorbic acid (0·07 mM) were added to retard catecholamine oxidation.

Radiochemicals and drugs

DL-[^{3}H]adrenaline hydrochloride (spec. act., 396 GBq $mmol^{-1}$; radioactive concentration 37 MBq ml^{-1}) was obtained from the Radiochemical Centre, Amersham. Indomethacin was obtained from Merck Sharp and Dohme. PGE_2 and the sodium salt of PGI_2 were supplied by Upjohn.

[^{3}H]Adrenaline was diluted in Krebs-Henseleit solution. Indomethacin was dissolved in 0·1 M Na_2CO_3 and diluted with Krebs-Henseleit solution for perfusion through the gland. PGE_2 was stored as a stock solution containing Na_2HPO_4 (0·07 M) and NaH_2PO_4 (0·03 M) at −20 °C. PGI_2 stock solutions were prepared daily by dissolving PGI_2 in ice-cold 0·01 M Na_2CO_3 (pH = 10). Final solutions of PGE_2 and PGI_2 were obtained by diluting the required amount in 50 mM Tris buffer (pH = 8 at 4 °C). PGE_2 and PGI_2 were perfused through the gland by means of a slow infusion pump which delivered the solution into the perfusion stream where the perfusion line enters the arterial vasculature of the gland, at the rate of 0·01 ml min^{-1}. The PGI_2 solution was changed for a fresh solution (at 4 °C) every 10 min, and at this pH and temperature it is calculated to have a half-life of 93 min (Cho & Allen 1978). This solution reached the adrenal gland within 20 s of being introduced into the perfusion stream.

Statistical analysis

The statistical significance of the results was determined by the Wilcoxon rank-sum test. The concentration dependence of the effect of PGE_2 was established by fitting a regression line to the relationship between the response and the logarithm of concentration by least squares method. The correlation coefficient was obtained and tested for a significant difference from zero using the Student's *t*-test. In all cases, differences were considered as significant if $P < 0.05$.

RESULTS

Effects of indomethacin

The characterization of the resting efflux of radioactivity and efflux of radioactivity induced by splanchnic nerve stimulation has been described previously

(Collett & Story 1982). We have shown that stimulation-induced efflux is comprised almost entirely of [^{3}H]adrenaline and that release is calcium-dependent.

In control experiments, the resting efflux of radioactivity before the first period of splanchnic nerve stimulation was 159 Bq min^{-1} (s.e.m. = 17, n = 5). Electrical stimulation of the splanchnic nerve resulted in an increase in the efflux of radioactivity from the preparation amounting to 328 Bq (s.e.m. = 80). The resting efflux before the second period of nerve stimulation was 65% (s.e.m. = 2) of the resting efflux before the first period of nerve stimulation, and the stimulation-induced efflux for the second period of nerve stimulation was 95% (s.e.m. = 3) of that of the first. The resting and stimulation-induced effluxes for the third period were 47% (s.e.m. = 3) and 76% (s.e.m. = 4) of the corresponding effluxes for the first period, respectively.

Indomethacin (3 and 30 μM), present before and during the second period of nerve stimulation, did not alter the resting efflux of radioactivity. Indomethacin also had no effect on stimulation-induced efflux compared to control experiments. The stimulation-induced efflux in the second period of splanchnic nerve stimulation as a percentage of that in the first period, was 101% (s.e.m. = 5, n = 5) in the presence of 3 μm indomethacin, and was 93% (s.e.m. = 4, n = 3) in the presence of 30 μM. The resting output of 6-oxo-$PGF_{1\alpha}$ from the gland was reduced by 40% (from $1{\cdot}5 \pm 0{\cdot}3$ to $0{\cdot}9 \pm 0{\cdot}2$ ng ml^{-1}, n = 6, $P < 0{\cdot}05$) by 3 μM indomethacin and by 86% (from $2{\cdot}5 \pm 0{\cdot}2$ to $0{\cdot}3 \pm 0{\cdot}02$ ng ml^{-1}, n = 5, $P < 0{\cdot}01$) by 30 μM indomethacin.

Effects of PGE_2 and PGI_2 in the presence of indomethacin

In the presence of indomethacin (3 μM), the resting efflux of radioactivity before the first period of splanchnic nerve stimulation was 153 Bq min^{-1} (s.e.m. = 19, n = 6). The stimulation-induced efflux evoked by the first period of nerve stimulation was 454 Bq (s.e.m. = 114). The resting and stimulation-induced effluxes for the second period were 61% (s.e.m. = 3) and 95% (s.e.m. = 3), and for the third period, 44% (s.e.m. = 3) and 81% (s.e.m. = 2) of the corresponding effluxes for the first period, respectively.

The effect of PGE_2 on the efflux of [^{3}H]adrenaline elicited by splanchnic nerve stimulation is shown in Fig. 1. PGE_2 caused a concentration-dependent decrease in stimulation-induced efflux. At PGE_2 concentrations of 30 nM and above, inhibition of stimulation-induced efflux persisted after removal of PGE_2 before the third period of nerve stimulation. The stimulation-induced effluxes for the third period of nerve stimulation after washout of PGE_2 are shown in Fig. 2.

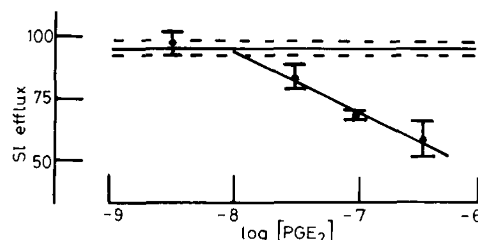

FIG. 1. The stimulation-induced (S-I) effluxes for the second period of nerve stimulation as a percentage of that for the first are plotted against the logarithm (base 10) of the PGE_2 concentration. Each point represents the mean of five experiments and the vertical lines represent the standard errors. The solid and broken horizontal lines represent the mean ± s.e.m. of stimulation-induced efflux in the presence of indomethacin alone. The line fitted to the data was obtained by linear regression analysis and the correlation coefficient of $-0{\cdot}65$ is significantly different from zero ($P < 0{\cdot}05$).

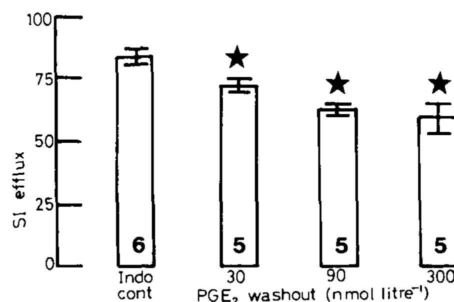

FIG. 2. The stimulation-induced (S-I) effluxes of radioactivity for the third period of nerve stimulation, after removal of PGE_2 from the perfusion system. The histograms represent the mean values of stimulation-induced effluxes in the third period expressed as percentages of that in the first. The vertical lines represent the standard errors and the number of experiments is shown inside each histogram. The asterisks denote a significant change in stimulation-induced efflux compared to experiments with indomethacin alone (Indo Cont); $P < 0{\cdot}05$ (Wilcoxon rank-sum test).

PGI_2, when present before and during the second period of nerve stimulation, was without effect on stimulation-induced efflux; the nerve stimulation-induced effluxes for the second period expressed as percentages of that for the first, at concentrations of 90, 300 and 600 nM, being 105% (s.e.m. = 7, n = 3), 90% (s.e.m. = 5, n = 3) and 87% (s.e.m. = 6, n = 3), respectively.

To ensure that PGI_2 retained activity during

perfusion of the adrenal gland, aliquots of the effluent were tested for inhibition of human platelet aggregation. In trial experiments, addition of 50 μl of the effluent (obtained 30 min or longer after beginning Krebs perfusion) to 0·5 ml of human platelet-rich plasma did not inhibit aggregation induced by ADP (20 μM). During adrenal perfusion with PGI_2 (90 nM), 50 μl aliquots of the effluent contained sufficient anti-aggregatory activity to completely inhibit ADP-induced aggregation, indicating that much less than 50% degradation of PGI_2 had occurred on passage through the perfusion circuit and the vasculature of the gland itself.

DISCUSSION

High concentrations of PGE_2 have previously been shown to decrease release of catecholamines from slices of the adrenal medulla of the ox (Boonyaviroj & Gutman 1979), rat (Boonyaviroj et al 1977) and man (Gutman & Boonyaviroj 1976). We have used a perfused preparation of the whole adrenal gland of the rabbit, in which the catecholamine stores were radiolabelled with [^{3}H]adrenaline (Collett & Story 1982). In this preparation PGE_2 dose-dependently inhibited the release of tritium label induced by electrical stimulation of the splanchnic nerve. We have previously established that the radioactivity released upon such stimulation consists almost entirely of [^{3}H]adrenaline. The inhibition of release of radioactivity that was observed after removing PGE_2 from the perfusion system (Fig. 2) may have been due to either the persistence of some intracellular process initiated by PGE_2, or to the persistence of PGE_2 in the gland as a result of slow metabolism.

In contrast with PGE_2, PGI_2 did not appear to have any effect on stimulation-induced efflux of [^{3}H]adrenaline from the adrenal gland. The failure of PGI_2 to influence efflux cannot be attributed to its degradation in the system for the effluent from the gland still contained appreciable PGI_2-like activity as measured by inhibition of platelet aggregation. In other isolated organs, PGI_2 also failed to influence release of [^{3}H]noradrenaline induced by stimulation of sympathetic post-ganglionic nerves, whereas PGE_2 generally has an inhibitory effect on transmitter release in the same preparations (Armstrong & Thirsk 1979; Hedqvist 1979; Smith et al 1982). Interestingly, the inhibitory effect of PGE_2 on adrenal catecholamine release can be correlated with observations on the specific binding of prostaglandins to particulate fractions of the adrenal medulla. Karaplis & Powell (1981) have shown that while high affinity binding sites for PGE_2 are present in this tissue, PGI_2 has considerably less affinity for any prostaglandin binding sites.

To determine whether endogenous prostanoids generated within the gland might inhibit catecholamine release, we investigated the effects of indomethacin. Indomethacin, in concentrations of 3 and 30 μM, which are known to inhibit or abolish prostanoid biosynthesis in other perfused tissues (Needleman 1978; Dusting et al 1981) and substantially reduce the output of 6-oxo-$PGF_{1\alpha}$ from this tissue, had no effect on stimulation-induced efflux of [^{3}H]adrenaline from the adrenal gland. This appears to conflict with findings in the anaesthetized cat (Feuerstein et al 1979, 1980) where release of adrenal catecholamines was induced by haemorrhage, and this response was much greater in cats treated with indomethacin. However, it is difficult to interpret this result because indomethacin also greatly increased the hypotension following haemorrhage, and baroreflexes would obviously be very different in the two situations.

Ramwell et al (1966) reported that prostaglandin-like material could be detected in the venous effluent from cat perfused adrenal glands following infusion of acetylcholine. However, our results in the rabbit isolated adrenal would suggest that endogenous PGE_2 is not present in sufficient quantities to exert an inhibitory action on catecholamine release from chromaffin granules. It remains possible, although in our view unlikely, that other unknown cyclo-oxygenase metabolites with a facilitatory action on catecholamine release could have masked the inhibitory effect of endogenous PGE_2 in our study. Moreoever, in view of the inconsistencies in the reported effects of prostaglandins on adrenal catecholamine secretion (see Introduction), the possibility that endogenous prostanoids might exert a modulatory influence on catecholamine release in species other than the rabbit cannot be ruled out.

In conclusion, release of [^{3}H]adrenaline from the rabbit adrenal gland is reduced by PGE_2 and unaffected by PGI_2. The lack of effect with indomethacin indicates that under physiological conditions it is unlikely that endogenous PGE_2 exerts a negative feedback action on catecholamine release from the rabbit adrenal gland. However, it is also possible that PGE_2, generated elsewhere in the cardiovascular system as a result of some pathological condition (such as immediate hypersensitivity reactions) and transported to the adrenal in arterial blood, could exert an inhibitory effect on adrenal catecholamine secretion.

Acknowledgements
This work was supported by grants (to G. J. D. and D. F. S.) from the National Heart Foundation of Australia.

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