Br. J. Pharmac. (1972), **44**, 699–710.

The actions of prostaglandins E_1 and $F_{2\alpha}$ on the perfused vessels of the isolated rabbit ear

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Summary

1. In the isolated rabbit ear vascular bed, perfused with Krebs solution, prostaglandins E_1 and $F_{2\alpha}$ produce dose-dependent, phentolamine-sensitive constrictions.

2. These are absent if the animal is pre-treated with reserpine or if the ear is denervated in advance.

3. If noradrenaline or vasopressin is added to the Krebs solution, vascular resistance is high and PGE_1 and $PGF_{2\alpha}$ produce vasodilatation which is unaffected by hyoscine or propranolol.

4. Perfusion with theophylline, with added ATP, ADP or 3′5′-AMP, or pre-treatment of the animal with stilboestrol antagonizes the dilator response to PGE_1 in the presence of noradrenaline, which may be reversed. Most of the responses to $PGF_{2\alpha}$ are reversed. These treatments elevate the level of 3′5′-AMP in tissues.

5. It is postulated that prostaglandins exert a regulatory action on 3′5′-AMP levels through inhibition of adenyl cyclase and/or phosphodiesterase and that the resulting rising or falling level of 3′5′-AMP determines the nature of the response by the smooth muscle to the released noradrenaline.

Introduction

The external ear of the rabbit consists of a cartilaginous skeleton, muscles inserted near its base, sensory nerves, and a richly innervated system of vessels supplying the overlying skin. When perfused it is a convenient model with which to study the effects of drugs on a predominantly cutaneous vascular bed. This preparation maintains a stable resistance over long periods of time (Al Tai & Graham, 1969, 1970, 1971a). The level of this resistance may be adjusted by addition of selected concentrations of (−)-noradrenaline or other vasoconstrictor to the perfusing fluid. The preparation is sensitive to vasoactive substances and gives reproducible results.

Prostaglandin E_1 (PGE_1) when injected intravenously lowers the arterial and pulmonary blood pressures of the anaesthetized rabbit (Bergstrom & von Euler, 1963; Horton & Main, 1963, 1965; Holmes, Horton & Main, 1963). $PGF_{2\alpha}$ is also a hypotensive agent in the rabbit (Anggard & Bergstrom, 1963) but is weaker and slower in onset than PGE_1.

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PGE_1 produces dilatation of the perfused vessels of the rabbit hind limb (Beck, Pollard, Kayaalp & Weiner, 1966). It may contract the relaxed smooth muscle of the spiral strip of rabbit aorta (Strong & Bohr, 1967) but in a concentration of 100 pg/ml relaxes the same preparation if the muscle has been pre-treated with noradrenaline.

PGE_1 increases cutaneous blood flow in cats (Horton & Main, 1963; Holmes *et al.*, 1963) and dogs (Nakano & McCurdy, 1967) but there is a lack of detailed studies of the effects of these substances on the blood vessels of the skin. The experiments described in this paper were designed to investigate them further.

Methods

Young N.Z. white rabbits of either sex weighing 2–3 kg were used throughout. The techniques of perfusion and recording have been described (Al Tai & Graham, 1971a & b; Sani, 1971). The effect of a drug is expressed as the percentage change in an index of peripheral resistance where peripheral resistance units (P.R.U.)=pressure in mmHg/peak flow in ml per min (1 mmHg≡1·333 mbar). If C=P.R.U. under control conditions and R=P.R.U. during response to a drug, $\triangle$%P.R.U.=(C—R/C) × 100 reflects the effect of the drug on the vascular bed (Al Tai & Graham, 1970). Values of P.R.U. measured during the response to a drug refer to its effect on peak flow. The perfusion fluid was Krebs solution of the following composition (mM): NaCl, 113; KCl, 4·7; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $NaHCO_3$, 25 and glucose, 11·5, gassed with 5% CO_2 in O_2 at 37° C. In some experiments the concentration of Ca^{2+} or of K^+ was doubled. Perfusion for 2 h preceded experimentation. This produces a constant low resistance (an atonic preparation). In many cases (−)-noradrenaline was added to the reservoir to produce a final concentration of 0·59 μM (0·1 μg/ml). This produces a constant high resistance (tonic preparation). In some preparations vasopressin (2 U/l.) was substituted for noradrenaline. A constant inflow pressure of 70 mmHg (93·31 mbar) was maintained in some cases and the resistance (in mmHg) and outflow (in ml/min) recorded; in the majority of experiments an approximately constant inflow rate of 2 ml/min was maintained by a roller pump (Quickfit 10 PP 60) and resistance and outflow recorded. Prostaglandins were administered by infusion from a suitable reservoir and a by-pass (Sani, 1971) or by injection in 0·1 ml vol of saline into the tubing near to the arterial cannula. Especial care was taken to ensure that separate syringes and needles were used for each dose of each prostaglandin. The time interval between injections was 15 min and between infusions 45 minutes. Drugs such as nucleotides, phentolamine and theophylline were dissolved in Krebs solution and added to the reservoir to make pre-determined concentrations of the base.

The drugs used were acetylcholine chloride, adenosine-3′5′-cyclic monophosphate (3′5′-AMP), adenosine-5′-diphosphate (ADP) and adenosine-5′-triphosphate (ATP), atropine sulphate, hyoscine hydrobromide, (−)-noradrenaline bitartrate, phentolamine mesylate, KCl (Analar), $CaCl_2$ (Analar), progesterone injection B.P., (±)-propranolol hydrochloride, reserpine, stilboestrol injection B.P., theophylline ethylenediamine, vasopressin B.P.

Solutions of prostaglandins were prepared with a microsyringe as follows: 1 mg PG was dissolved in 0·1 ml of cold 95% ethanol in water and made up to

1 ml with a cold solution containing 0·2 mg anhydrous sodium carbonate per ml of deionized water. Aliquots of 0·1 ml were sealed in ampoules and stored at −20° C. Dilutions were made shortly before experimentation and the solutions in 0·9% NaCl kept on a salt and ice mixture.

Prepared animals

Injection of drugs

Six rabbits were injected with 0·2 mg of reserpine subcutaneously daily for 3 days before perfusion of the ears. Rabbits of either sex (10 female and 6 male) were injected intramuscularly with stilboestrol, 0·5 mg followed by 0·1 mg daily for a further 3–10 days. The animals were killed on the 4th or the 12th day. Four rabbits (female only) received progesterone 5 mg intramuscularly daily for 12 days; four females received both hormones, i.e. the stilboestrol as scheduled and progesterone from the 5th day until the 11th day.

Surgical preparation

(1) Under anaesthesia induced by pentobarbitone sodium (30 mg/kg, i.v.) bilateral ovariectomy was performed on 20 virgin rabbits of 2–3 kg weight and the animals were allowed to recover. Of these, 3 were not given drugs, 7 received the schedule of injections of stilboestrol and 10 the schedule of stilboestrol and progesterone.

(2) In 6 female rabbits given atropine (1 mg/kg) and anaesthetized with ether and pentobarbitone sodium (30 mg/kg) the left superior cervical ganglion was removed, a segment of 1 cm length of the great auricular nerve removed and the central ear artery stripped of its perivascular connective tissue for a distance of 1 to 1·5 cm, with recovery. Both ears were used 10 days later.

Results

Responses to PGE_1 and $PGF_{2\alpha}$

Constant pressure

In the atonic vessels perfused at constant pressure, injected PGE_1 had no effect whereas infused PGE_1 produced a 10–20% constriction at a dose level of 10 ng/ml after a delay of 10–15 minutes. $PGF_{2\alpha}$ was only used in a few experiments by constant pressure technique. It was vasoconstrictor. In the high tone preparation (noradrenaline 0·59 μM) infused PGE_1 produced a dose-related dilatation in concentrations of 100 pg–10 ng/ml. This effect, unlike the preceding one, persisted after the infusion of PGE_1 had ceased.

Constant flow

In the atonic preparation (initial pressure 15–30 mmHg) injection or infusion of PGE_1 was vasoconstrictor. In 3 of 6 preparations tested, PGE_1 in a concentration of 100 pg/ml produced a brief and marginally detectable constriction of $+5 \pm 2$ Δ%P.R.U.; the regularly effective concentration was 10 ng/ml which produced a constriction of $+41 \pm 5{\cdot}9$ Δ%P.R.U. within 2 minutes. This effect was converted by 15 min perfusion with phentolamine (1 μg/ml) to a dilator response of $-14 \pm 1{\cdot}7$

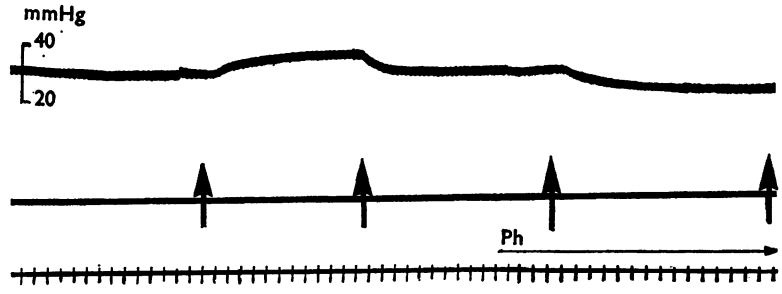

FIG. 1. Isolated rabbit ear perfused with Krebs solution at 37° C, 2 ml/min constant flow. Infusion with 10 ng/ml PGE_1 for 13 min (↑↑) causes a constrictor response of +41±5·9 △% P.R.U. Phentolamine 1 μg/ml (Ph→) abolishes this constriction and reveals a dilatation. Pressure in mmHg. Time in minutes.

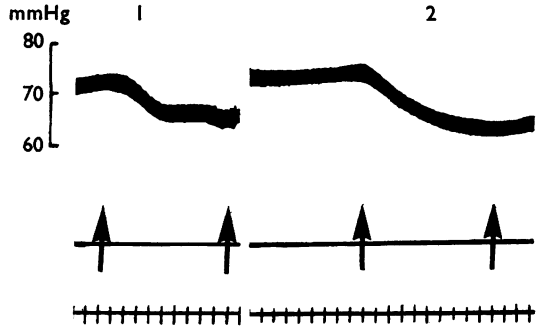

FIG. 2. Isolated rabbit ear perfused with Krebs solution containing noradrenaline 0·59 μM at 37° C and 2 ml/min constant flow. The response to infused PGE_1 (↑↑) is dilator. 1=1 pg/ml; 2=10 ng/ml. Pressure in mmHg. Time in minutes. The dilator effect is of moderate intensity and is prolonged.

TABLE 1. *The dilator response to injections of PGE_1 in the vascular bed of the rabbit ear perfused with noradrenaline 0·59 μM in Krebs solution at 2 ml/min*

		Doses of PGE_1			
Perfusion fluids	n	10 ng	1 μg	10 μg	
		Mean responses to injected PGE_1			
Kr+NA	10	18±1·9	39±1·6 (control)	50±1·5	% Diff. from control
Kr+NA+theophylline	6	3±1	16±2·4	30±2·7	−59*
Kr+NA+ATP	6	1±0·4	8±2·1	12±1·4	−79*
Kr+NA+ADP	7	1±0·6	13±2	25±3	−67*
Kr+NA+cyclic AMP	6	1±0·6	13±2·3	28±3·4	−67*
Kr+NA+double K^+	6	12±1·6	33±1·5	42±1·2	−15 NS
Kr+NA+double Ca^{2+}	7	13±2·2	33±3	44±2	−15 NS
Kr+NA+double (Ca^{2+})+ATP	4	2±0·5	13±1·4	23±2·4	−67*

Kr=Krebs solution; NA=noradrenaline 0·59 μM; the additives were: theophylline 1 μg/ml; ATP, ADP, and cyclic AMP 10 μg/ml. *=$P<0·001$; NS=$P>0·05$. Mean responses are expressed in △% P.R.U.±S.E. The % difference is stated for one dose level only of PGE_1.

Δ%P.R.U. (see Fig. 1). This dilator response was not affected by hyoscine or propranolol in the perfusion fluid in concentrations of 1 μg/ml. The vasoconstrictor response was abolished by pretreatment of the animals with injection of reserpine, and it was absent in denervated ears. A small dilator response was then revealed.

The effect of $PGF_{2\alpha}$ in this preparation was very similar to that of PGE_1, the differences being solely quantitative. In the atonic preparation an injection had no effect or produced a slight constriction; infusion was most effective in a concentration of 10 ng/ml and caused a phentolamine-sensitive constriction which was absent in denervated ears or ears from animals pretreated with reserpine.

In the tonic preparation (noradrenaline 0·59 μM, pressure 70–100 mmHg; or vasopressin 2 U/l, which settles at approximately 60 mmHg) both injection and infusion of PGE_1 produced a dose-related dilatation (see Fig. 2 and Table 1). The range of effective injections was 10 ng to 10 μg and of infusions of 1 pg to 10 ng/ml. Perfusion with phentolamine 1 μg/ml reduced the initial pressure in the noradrenaline-perfused preparation to about 40 mmHg but infusions of PGE_1 at 10 ng/ml still had a dilator effect, though much reduced. Hyoscine, propranolol, reserpine or denervation (which sensitizes to noradrenaline) did not radically affect this dilatation of the tonic vessel by PGE_1. The response to $PGF_{2\alpha}$ is less consis-

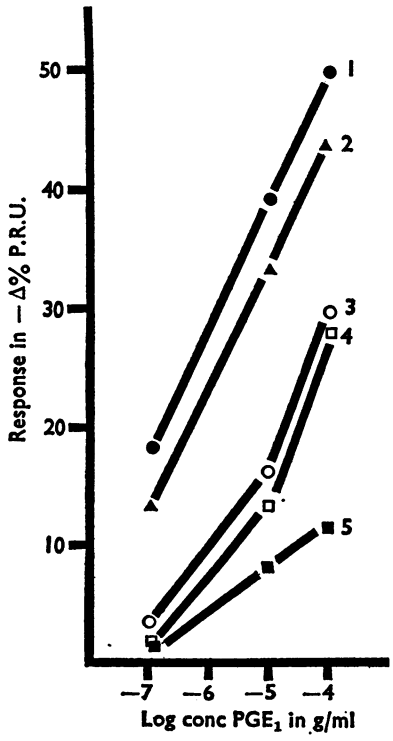

FIG. 3. Isolated rabbit ear perfused with Krebs solution containing noradrenaline 0·59 μM at 37° C and a constant flow of 2 ml/min. Injected PGE_1. Ordinates Δ% P.R.U. (i.e. dilator responses), abscissae log conc. PGE_1 in g/ml; standard injection of 0·1 ml. Each point is the mean of 10 observations. 1=●=control; 2=▲=doubled Ca^{2+}; 3=○=theophylline (1 μg/ml); 4=□=3′5′-AMP 10 μg/ml; 5=■=ATP added to the perfusing fluid. All treatments shift the dose-response curve to the right; ATP considerably reduces the response and alters the slope.

tent, particularly if it is injected. By this route 10 ng to 10 μg caused a small dilatation, sometimes followed by an increase in perfusion pressure of a few mmHg. The dilatation was not consistently dose related. Infusions of $PGF_{2\alpha}$ of 1 pg to 100 pg/ml had no effect but 10 ng to 100 ng/ml were constrictor.

Effects of theophylline and of nucleotides on the responses to prostaglandins

Concentrations of 1 μg/ml theophylline in the perfusing fluid (noradrenaline 0·59 μM) gave rise to an increase in resistance of 10–30 mmHg; lesser concentrations had no effect. The dose-response curve (dilator response) to injected PGE_1 was moved to the right but the slope remained substantially the same (see Fig. 3). The dilator response to infusion of 10 ng/ml was reduced; the response to infusion of 1 pg to 100 pg/ml was reversed i.e. changed to a constriction (see Fig. 4). The effects of theophylline on PGE_1 were more apparent after 90 min perfusion than after 30 min perfusion; the effects of theophylline on $PGF_{2\alpha}$ were similar i.e. conversion of the dilator response to injections to a constrictor response (of some +20Δ%P.R.U.).

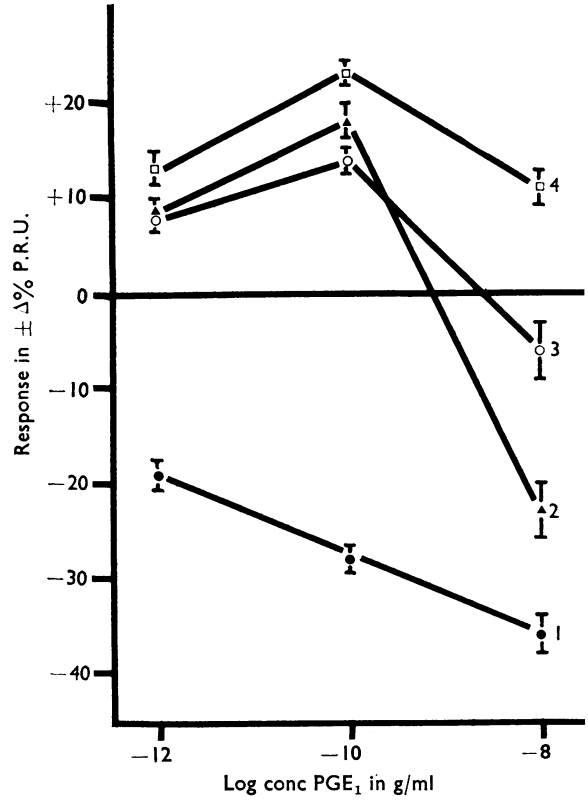

FIG. 4. Isolated rabbit ear perfused with Krebs solution containing noradrenaline 0·59 μM at 37° C and a constant flow of 2 ml/minute. Infused PGE_1. Ordinates ±Δ% P.R.U. (i.e. dilator responses below and constrictor responses above the zero line), abscissae log conc. PGE_1 in g/ml. Each point is the mean of 10 observations. 1=●=control; 2=▲=theophylline 1 μg/ml; 3=○=double Ca^{2+}; 4=□=3′5′-AMP 10 μg/ml added to the perfusing fluid. All 3 treatments reverse the response to infused PGE_1 1 pg and 100 pg/ml. 3′5′-AMP also reverses the response to PGE_1 10 ng/ml.

Adenosine 3′5′-monophosphate in the relatively high concentration of 10 μg/ml in the perfusion fluid potentiated the pressor effect of infused noradrenaline. The dose-response curve (dilator) to injected PGE_1 was shifted to the right (see Fig. 3); the effects of infusions of PGE_1 were reversed, i.e. converted to constriction (see Fig. 4). The effect of injections (see Fig. 5) and infusions of $PGF_{2\alpha}$ were also reversed. Lesser concentrations of 3′5′-AMP did not alter the responses to prostaglandins. The nucleotides ATP and ADP caused similar changes. ATP in concentrations of 1–10 μg/ml lowered the tone of the noradrenaline-perfused circulation. The basic level of resistance (approx. 100 mmHg pressure) was restored by doubling the concentration of noradrenaline in the perfusing fluid. Dilator responses produced by injection of 10 ng of acetylcholine were not appreciably altered by the presence of ATP. In a concentration of 10 μg/ml ATP after 90 min shifted the dose-response curve to injected PGE_1 to the right and greatly reduced or abolished the dilator response to infusion of it. In 2 out of 10 experiments infusion of 100 pg/ml PGE_1 gave rise to a +20△% P.R.U. constriction. The dilator effect of $PGF_{2\alpha}$ (injection of 1 μg or infusion of 100 pg/ml) was altered to a constrictor response by the addition of ATP 10 μg/ml to the Krebs solution containing noradrenaline. ADP in the same concentration (10 μg/ml) perfused for 90 min had much less effect than ATP in lowering noradrenaline-maintained tone. It effectively reduced the dilator response to PGE_1 and converted that to $PGF_{2\alpha}$ into constriction.

All responses reverted to their initial type and size on further perfusion of the ear vessels with Krebs solution free of additives other than noradrenaline.

Effects of increased calcium or potassium ion

The concentration of Ca^{2+} or of K^+ in the perfusing fluid (noradrenaline 0·59 μM in Krebs) was doubled in each of 7 experiments. The basic perfusion pressure was markedly elevated as a result. In the presence of high Ca^{2+} levels the dilator

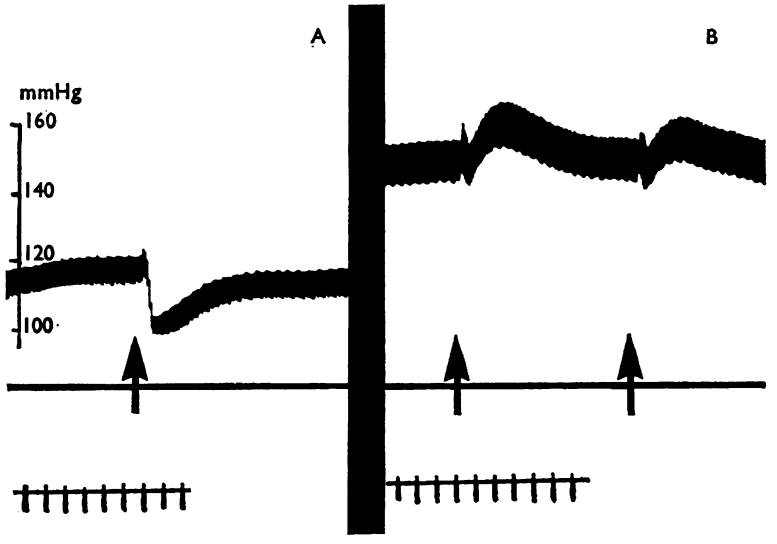

FIG. 5. Isolated rabbit ear perfused with Krebs solution containing noradrenaline 0·59 μM at 37° C and constant flow of 2 ml/min; effects of injection of $PGF_{2\alpha}$ 1 μg. A—perfused with Krebs—noradrenaline. B—effect of adding 3′5′-AMP, 10 μg/ml. Pressure in mmHg. Time in minutes.

effects of injection of PGE_1 were not reduced significantly (see Fig. 3 and Table 1). Dilatation due to perfusion with PGE_1 10 ng/ml was reduced: that due to 1 pg and 100 pg/ml was reversed (constrictor; see Fig. 4). $PGF_{2\alpha}$ infusions at 10 ng/ml were reversed (constrictor) and injections of 1 to 10 μg also produced a small constrictor response. The return to baseline of the resistance after perfusion with PGE_1 had ceased was quicker and more complete when the Ca^{2+} level was high than when the normal level of Ca^{2+} was present. The effects of increased K^+ level on the responses to PGE_1 were less definite. Injections of PGE_1 produced an abrupt, brief and less intense dilatation. Infusions of 1 pg or 100 pg/ml produced variable responses but 10 ng/ml was dilator.

Addition of ATP to either modified perfusion fluid had the same antagonistic effect on the response to PGE_1 as when the ionic composition of the Krebs solution was unaltered.

Effects of sex hormone treatment on the responses to prostaglandin

No differences were detected in the responses of ovariectomized or of non-ovariectomized animals, injected with stilboestrol, progesterone or the mixed regimen, in the responses of the perfused ear to prostaglandins.

Ovariectomized animals not treated with sex hormones did not vary greatly in response as compared with non-ovariectomized untreated animals if they were used within 7 days of operation.

Atonic preparation

A few experiments were carried out with constant flow perfusion with Krebs solution. Infusions of PGE_1 and of $PGF_{2\alpha}$, vasoconstrictor as in untreated animals, were reduced in efficacy by progesterone pretreatment and enhanced by stilboestrol pretreatment. The constrictions were phentolamine-sensitive, and small dilator responses (abolished by ATP) were revealed.

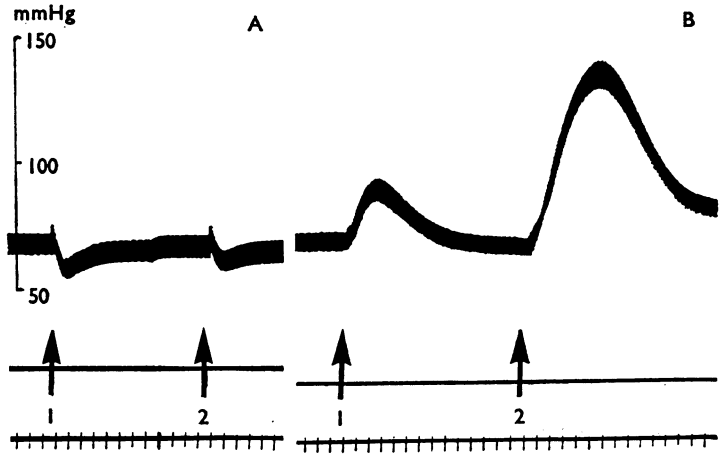

FIG. 6. Isolated rabbit ear perfused with Krebs solution containing noradrenaline 0·59 μM at 37° C and constant flow of 2 ml/minute. Effect of injections of $PGF_{2\alpha}$. 1=1 μg, 2=10 μg. Panel A, untreated animal—dilator responses. Panel B, animal injected with stilboestrol for 11 days—constrictor responses. Pressure in mmHg. Time in minutes.

Tonic preparation (vasopressin 2 U/l. or noradrenaline 0·59 μM)

Both of these vasoconstrictor agents raise the basic resistance. Pretreatment of the animal with stilboestrol sensitizes the vessel to this action; progesterone antagonizes it. PGE_1 and $PGF_{2\alpha}$ by infusion are dilators in normal intact animals. After stilboestrol pretreatment (minimum 3 days in the female, 6 in the male) the response to PGE_1 1 pg and 100 pg/ml was reversed (see Fig. 6) and that to 10 ng/ml much reduced; 10 days treatment with stilboestrol elicited pressor responses to all infusions of PGE_1 and $PGF_{2\alpha}$. The optimal concentration of PGE_1 was then 100 pg/ml. A similar enhancement of vasoconstrictor responses followed the combined pretreatment with stilboestrol and progesterone, and in preparations from rabbits taken late in pregnancy (24th day). These constrictor responses are phentolamine-sensitive, and are enhanced by the presence of propranolol (1 μg/ml) in the perfusion fluid. ATP (10 μg/ml in Krebs solution for 90 min) had the same effect in stilboestrol-treated animals as in normal animals.

Discussion

The constrictor effect of PGE_1 and $PGF_{2\alpha}$ on the vessels perfused with Krebs solution is abolished by pretreatment with reserpine, denervation or phentolamine. It may be due to release of noradrenaline from adrenergic nerves. Such a conclusion is in agreement with the finding of Graham & Katib (1967) that a congener of dibenamine (SY 28) antagonized the stimulant action of PGE_1 and of noradrenaline on the isolated vas deferens of the guinea-pig. The small dilatation which is revealed by these treatments is not abolished by hyoscine or propranolol. In the isolated perfused mesenteric arterial bed of an unstated species Tobian & Viets (1970) found that PGE_1 in concentrations which were otherwise inactive doubled the constrictor response to infused noradrenaline, in contrast to an antagonism demonstrated *in vivo*. They postulated the existence of a 'plasma component' (perhaps a mutual binding site) present *in vivo* and absent *in vitro*. The present experiments refute this postulate since dilatation is consistently observed in a thoroughly washed vascular bed perfused with PGE_1-noradrenaline in Krebs solution. Many authors have described a synergism and/or antagonism between prostaglandins and catecholamines in a variety of species and tissues, e.g. the guinea-pig seminal vesicle (Goldblatt, 1935) or the rat vas deferens (Chambers & Pickles, 1958), guinea-pig seminal vesicle (Eliasson & Risley, 1967) and vas (Graham & Katib, 1967). Holmes *et al.* (1963) reported that PGE_1 inhibited contractions of rabbit vas deferens induced by adrenaline. The most extensive report is that of Clegg (1966) which makes it clear that PGE_1 and $PGF_{2\alpha}$ may initially potentiate and usually subsequently antagonize responses to catecholamines (whether by contraction or relaxation) of a variety of smooth muscles from a number of species.

The dilator effect of prostaglandin (in particular of PGE_1) on the vessel perfused with noradrenaline or vasopressin is in line with the reported action of PGE_1 in antagonizing the pressor effect of noradrenaline (Carlson & Oro, 1966), of vasopressin and of angiotensin (Holmes *et al.*, 1963; Weeks & Wingerson, 1964) by a direct vasodilator action on many vascular beds. The rabbit ear vascular bed probably contains β-adrenoceptors. Propranolol enhances the constrictor response to infused isoprenaline in the noradrenaline-perfused ear (Al Tai & Graham,

1971b) and abolishes the dilatations revealed by phentolamine. Nevertheless propranolol does not affect the dilatations caused by PGE_1 in the noradrenaline-perfused ear. The mechanism must differ from that of the β-adrenoceptor, which is held to be related to the activity of adenyl cyclase and the level of 3′5′-AMP in cardiac tissue (Sutherland, Robison & Butcher, 1968).

The most interesting feature of the present report is the reversal of the constrictor response to PGE_1 given by the low tone preparation when noradrenaline or vasopressin is added to the Krebs solution, and the restoration of constriction when a number of treatments are given. All of these tend to raise the level of 3′5′-AMP, e.g. perfusion with the precursors ATP, ADP, AMP (in that order of potency), stilboestrol injections (Szego & Davies, 1967), or inhibition of phosphodiesterase by theophylline (Sutherland & Rall, 1960). Noradrenaline has been shown to stimulate adenylcyclase activity in a number of systems and to antagonize it in others (Butcher, Robison & Sutherland, 1970); vasopressin is thought to stimulate it (Orloff & Handler, 1967). Intracellular ATP, pyrophosphate (a product of the adenyl cyclase reaction) and citrate inhibit phosphodiesterase (Cheung, 1967). The high tone of the vessel in the presence of noradrenaline or vasopressin and the loss of the dilator response to PGE_1 when 3′5′-AMP is further increased implies that high levels of 3′5′-AMP are correlated with vasoconstriction and that PGE_1 reduces these levels. One may postulate inhibition of adenyl cyclase, stimulation of phosphodiesterase or another mechanism which reduces the level or blocks the utilization of 3′5′-AMP. Falck, Owman, Rosengren & Sjöberg (1969) showed that oestrogen causes an increase in the catecholamine content of uterine muscle and progesterone reduces it. Bartelstone, Nasmyth & Telford (1967) reached the conclusion that noradrenaline and 3′5′-AMP are synergists in causing contraction of the myometrium. The same conclusion would seem to apply to the vessels of the rabbit ear.

Pickles (1967, 1969) has suggested that prostaglandins may act as carriers of Ca^{2+} in the cell, acting at some point after agonist-receptor activation. Eagling, Lovell & Pickles (1971) noted synergism between Ca^{2+} in the bathing fluid and contractions of the myometrium induced by PGE_1. We have noted synergism between Ca^{2+} in the perfusing fluid and noradrenaline-induced vasoconstriction, and antagonism to or reversal of dilatations induced by infused PGE_1. Artificially raised K^+ level had a similar synergistic action with noradrenaline but little effect on the responses to PGE_1 or $PGF_{2\alpha}$.

We postulate at least two main actions of PGE_1 on the perfused ear vessels of the rabbit:

(1) Prostaglandin E_1 releases noradrenaline

This accounts for the constrictor response in low tone preparations. The response to this depends on the level of 3′5′-AMP in the tissue and may be altered by appropriate treatment. If the level rises as a result of noradrenaline action contraction of the smooth muscle ensues.

(2) Prostaglandin E_1 acts as a regulator of adenylcyclase-phosphodiesterase balance

PGE_1 may stimulate or inhibit adenyl cyclase. In the high tone preparation **3′5′-AMP levels are probably high. Release of noradrenaline by PGE_1** would have little additional effect; inhibition of adenyl cyclase or stimulation of phospho-

diesterase by PGE_1, if superimposed on the background of continuing noradrenaline stimulation, might produce a decline in 3'5'-AMP level associated with relaxation of the smooth muscle. $PGF_{2\alpha}$ may stimulate adenyl cyclase or inhibit phosphodiesterase. The regulator function may alternate as the level of 3'5'-AMP shifts, tending to restrict the degree of variation.

One of us (S. A. Al Tai) is a Gulbenkian Scholar of the Department of Pharmacology, University of Baghdad, Iraq. We are grateful for the gift of PGE_1 from Messrs Upjohn Ltd., and to Professor D. A. van Dorp for crystalline PGE_1 and $PGF_{2\alpha}$. We are grateful to Professor V. R. Pickles for the hospitality given to one of us (S. A. Al Tai) during a period of laboratory removal.

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(*Received November* 2, 1971)