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PROSTAGLANDIN $F_{2\alpha}$ REDUCES THE ALGESIC EFFECT OF BRADYKININ BY ANTAGONIZING THE PAIN ENHANCING ACTION OF ENDOGENOUSLY RELEASED PROSTAGLANDIN E

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1 The isolated perfused ear of the rabbit connected to the body only by its nerve, was used to investigate the influence of prostaglandin $F_{2\alpha}$ on the algesic effect of bradykinin and acetylcholine.

2 Bradykinin and acetylcholine, following intra-arterial injection into the isolated perfused ear elicited a dose-related reflex fall in blood pressure due to stimulation of paravascular pain receptors (= algesic effect).

3 Infusion of prostaglandin $F_{2\alpha}$ (0.1 to 1 ng/ml) into the rabbit ear reduced the algesic effect of bradykinin but not that of acetylcholine.

4 The onset of the reflex fall in blood pressure by bradykinin but not that by acetylcholine was delayed by infusion of prostaglandin $F_{2\alpha}$ into the ear.

5 Infusion of prostaglandin E_1 into the rabbit ear led to an enhancement of the algesic effect of bradykinin and acetylcholine. Enhancement of both effects was abolished by infusion of prostaglandin $F_{2\alpha}$.

6 During inhibition of the endogenous synthesis of prostaglandins (mainly E-type) by indomethacin, a low concentration of prostaglandin $F_{2\alpha}$ no longer reduced the algesic effect of bradykinin. However, a high concentration of $F_{2\alpha}$ continued to enhance the effect of bradykinin and acetylcholine.

7 Prostaglandin $F_{2\alpha}$ influenced neither the brief reduction in venous outflow produced by bradykinin nor the brief increase in venous outflow caused by acetylcholine.

8 The results suggest that prostaglandin $F_{2\alpha}$ does not directly reduce the effect of bradykinin but inhibits the enhancement of its algesic effect produced by prostaglandin E that is released endogenously by bradykinin. That the algesic effect of acetylcholine is not reduced by prostaglandin $F_{2\alpha}$ is in keeping with its releasing very little endogenous prostaglandin E.

Introduction

Prostaglandins of the E and F series exert opposite effects in a variety of situations but so far nothing has been reported of opposing effects on 'pain receptors', represented by sensory nerve endings. However, there are a few reports concerning a pro-inflammatory effect of E prostaglandins and an anti-inflammatory effect of F-prostaglandins: prostaglandin E_1, given intradermally in rats, potentiated the increase in vascular permeability induced by bradykinin while prostaglandin $F_{2\alpha}$ inhibited the response (Willoughby, 1968; Thomas & West, 1973; 1974). Furthermore, the increased vascular permeability caused by intradermal prostaglandins E_1 and E_2 in rats was reduced by prostaglandin $F_{2\alpha}$ (Crunkhorn & Willis, 1969; 1971).

In earlier experiments on vascular pain receptors we used the rabbit isolated perfused ear method of Mirzojan & Doblatian (1955) and Mietkiewski (1956). We found that intra-arterial injection of algesic substances into the isolated perfused ear of the rabbit stimulated paravascular pain receptors, elicited action potentials in the great auricular nerve and produced a reflex fall in arterial blood pressure. Bradykinin and acetylcholine were found to be the most effective substances (Juan & Lembeck, 1973; 1974b). Prostaglandin E_1 strongly enhanced the algesic effect of all substances used and most that of bradykinin (Juan & Lembeck, 1974b). Prostaglandin E_2 was less active (Lembeck & Juan, 1974). Our findings with bradykinin and E prostaglandins confirmed the results of other authors (Ferreira, Moncada & Vane, 1973) on other preparations (see Discussion section).

In this paper we describe the opposing effects of E and F prostaglandins on the algesic effect of bradykinin and acetylcholine.

Methods

Rabbit ear preparation separated from the systemic circulation

Details of the method have been described earlier (Juan & Lembeck, 1974b). Rabbits of either sex (2.3–3.2 kg) were anaesthetized with pentobarbitone (35 mg/kg). An ear was perfused via its artery; the flow rate was adjusted by pressure to 5 ml/min (Tyrode solution bubbled with 95% O_2 and 5% CO_2, 37°C). The ear was left connected to the body by the great auricular nerve only. Injections of bradykinin and acetylcholine into the arterial inflow of the ear elicited a reflex fall in systemic arterial pressure of the lightly anaesthetized animal by stimulation of paravascular pain receptors. The fall in blood pressure, which in contrast to the rise in blood pressure in the dog after knee-joint stimulation (Ferreira, Moncada & Vane, 1973) was dose-related, was used as a quantitative parameter for the algesic effect. Blood pressure was measured in a carotid artery by a Statham pressure transducer P 23 D b and monitored with a Beckman type R 411 dynograph. Some ear preparations did not respond to bradykinin or became insensitive during the first 30 to 60 min of the experiment; these were discarded.

The venous outflow was measured with an electronic drop recorder connected to a Beckman type R 411 dynograph and changes were expressed as percentages of initial values.

Outline of the experimental procedure

Bradykinin and acetylcholine were injected into the arterial inflow cannula of the ear in 2 or 3 different quantities at the beginning of the experiments to obtain the control values. Thereafter, prostaglandin $F_{2\alpha}$ was infused into the ear in three concentrations (0.1, 1.0 and 10.0 ng/ml) for more than 60 minutes. The injections of bradykinin and acetylcholine were repeated during the infusion at constant intervals.

In another series of experiments bradykinin and acetylcholine were injected in two different amounts (bradykinin: 30 ng and 100 ng; acetylcholine: 3 μg and 10 μg). The lower amounts of each produced trivial reflex falls in blood pressure. Then infusion of 10.0 ng/ml prostaglandin E_1 was started and maintained during the whole experiment. Prostaglandin E_1 enhanced the algesic effect of bradykinin and acetylcholine as reported earlier (Juan & Lembeck, 1974b). About 30 min after the start of the infusion the enhancement of the effects of bradykinin and acetylcholine was almost maximal. These enhanced effects (C_E in Figure 3) served now as controls for the responses following additional infusions of prostaglandin $F_{2\alpha}$. Thirty-five minutes after the start of the infusion of prostaglandin E_1 an infusion of 50 ng/ml prostaglandin $F_{2\alpha}$ was given in addition to that of prostaglandin E_1 and maintained for 65 min (Figure 3). The injections of bradykinin and acetylcholine were repeated during the influence of prostaglandin $F_{2\alpha}$.

In further experiments prostaglandin $F_{2\alpha}$ and E_1 were injected in single doses 2 min before the injection of bradykinin or acetylcholine to obtain the control values. The injections were repeated during infusion with indomethacin 1 μg/ml for at least 30 minutes.

Statistical significance between means (against controls) was assessed by the paired *t* test.

Drugs

The following drugs were used: acetylcholine chloride (Becker, Vienna); synthetic bradykinin (Sandoz, Basel) and prostaglandins E_1 and $F_{2\alpha}$ (Upjohn Co., Kalamazoo). All drug concentrations are quoted in terms of the base or acid.

Results

Influence of prostaglandin $F_{2\alpha}$ on the algesic effect of bradykinin and acetylcholine

During infusion of 0.1 ng/ml prostaglandin $F_{2\alpha}$ (Figure 1) the algesic effect of bradykinin was reduced significantly ($P < 0.01$) and 1.0 ng/ml prostaglandin $F_{2\alpha}$ caused even greater reduction (Figure 2). During infusion of the high concentration of 10.0 ng/ml prostaglandin $F_{2\alpha}$, however, the algesic effect of bradykinin was hardly reduced at all. After the end of the infusion of prostaglandin $F_{2\alpha}$, the effect of bradykinin was restored slowly, taking more than 100 minutes.

In contrast the effect of acetylcholine was never reduced by prostaglandin $F_{2\alpha}$ in any of the three concentrations used (Figures 1 and 2).

Interaction between prostaglandin E_1 and $F_{2\alpha}$

During the concomitant infusion of prostaglandin E_1 (10 ng/ml) and prostaglandin $F_{2\alpha}$ (50 ng/ml) into the ear (Figure 3), the prostaglandin E_1-enhanced effects of bradykinin and of acetylcholine were reduced significantly ($P < 0.01$). After terminating the infusion of prostaglandin $F_{2\alpha}$, prostaglandin E_1 was infused for a further period. Responses to both bradykinin and acetylcholine resumed their previous magnitude (C_E Figure 3) during a recovery period of 80 to 120 min for bradykinin and about 60 min for acetylcholine.

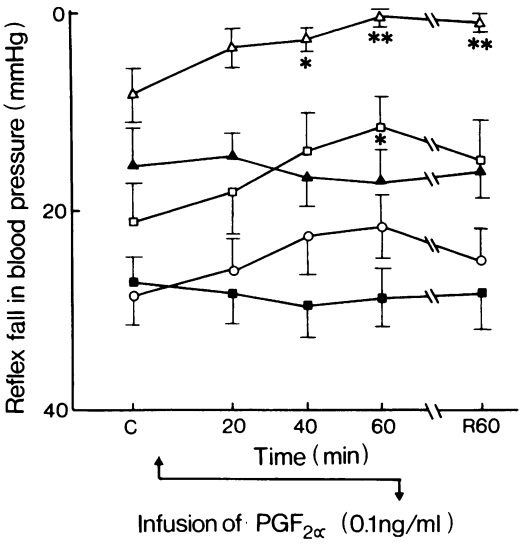

Figure 1 Rabbit ear separated from the systemic circulation. Influence of infusion of 0.1 ng/ml prostaglandin $F_{2\alpha}$ on reflex fall in mean arterial blood pressure produced by bradykinin: 0.1 μg (△), 0.3 μg (□) and 1.0 μg (○) and acetylcholine: 10 μg (▲) and 30 μg (■). Controls = C. R60 = recovery, 60 min after the end of infusion. Each point represents the mean of results from 6 rabbits. Vertical bars represent s.e. mean. $*P < 0.05$; $**P < 0.01$.

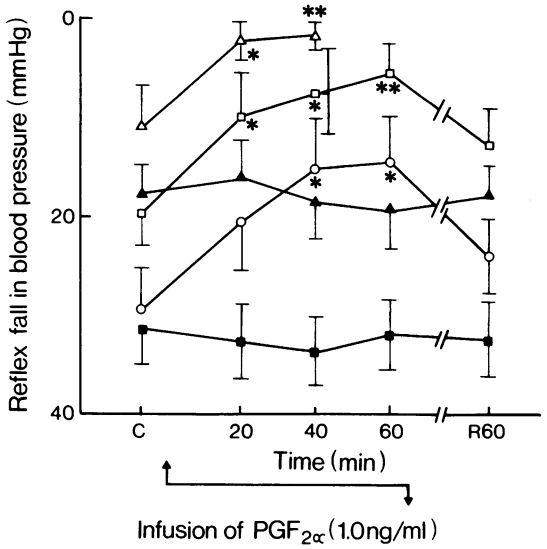

Figure 2 Rabbit ear separated from the systemic circulation. Influence of infusion of 1.0 ng/ml prostaglandin $F_{2\alpha}$ on reflex fall in mean arterial blood pressure produced by bradykinin: 0.1 μg (△), 0.3 μg (□) and 1.0 μg (○) and acetylcholine: 10 μg (▲) and 30 μg (■). Controls = C. R60 = recovery, 60 min after the end of infusion. Each point represents the mean of results from 5 rabbits. Vertical bars represent s.e. mean. $*P < 0.05$; $**P < 0.01$.

Influence of prostaglandin E_1 and $F_{2\alpha}$ on the delay time of the pain reflex response

The delay time of the pain reflex response (time between injection and onset of reflex fall in blood pressure) is longer for bradykinin than for acetylcholine (Juan & Lembeck, 1974b).

During infusion of prostaglandin $F_{2\alpha}$, the delay of the response to acetylcholine remained unchanged while that of the response to bradykinin increased significantly ($P < 0.01$) (Figure 4).

In another series of experiments the delays of the response to acetylcholine and bradykinin were unchanged by infusion of 10 ng/ml prostaglandin E_1. However, when in addition to prostaglandin E_1, prostaglandin $F_{2\alpha}$ (50 ng/ml) was also infused into the ear, the delay of the response to bradykinin was again significantly increased while that of the response to acetylcholine was not changed significantly (Figure 4).

Influence of prostaglandin E_1 and $F_{2\alpha}$ on the vasoactivity of bradykinin and acetylcholine

When bradykinin was injected intra-arterially into the rabbit ear, the venous outflow showed a shortlasting decrease. Following injection of small amounts of acetylcholine (10 μg), the venous outflow slightly increased.

In our experiments the perfusion rate was kept constant by inflow pressure adjustment so that a possible vasoconstriction produced by infusion of prostaglandin $F_{2\alpha}$ may have been compensated for without recognition. Therefore, we did not measure the influence of prostaglandin $F_{2\alpha}$ on venous outflow but only its influence on the shortlasting response to injected bradykinin and acetylcholine. The vasodilator effect of 10 μg acetylcholine was slightly increased during infusion of prostaglandin E_1 and $F_{2\alpha}$, separately or together. The vasoconstrictor effect of bradykinin was not altered during infusion of prostaglandin $F_{2\alpha}$, was reduced significantly ($P < 0.05$) during infusion of prostaglandin E_1 and was slightly reduced during the infusion of both prostaglandins together.

Influence of prostaglandin $F_{2\alpha}$ on the algesic effect of bradykinin and acetylcholine in the presence of indomethacin

A single injection of 0.1 μg prostaglandin $F_{2\alpha}$ 2 min before the injection of bradykinin (0.3 μg) significantly reduced the algesic effect of bradykinin (Figure 5). The effect of acetylcholine (10 μg) was again not influenced. During infusion of indomethacin 1 μg/ml the effect of bradykinin became attenuated so that larger amounts were required to produce the same

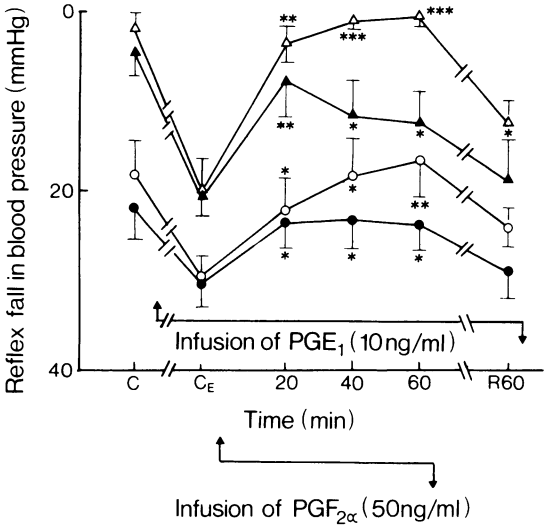

Figure 3 Rabbit ear separated from the systemic circulation. Reflex fall in mean arterial blood pressure elicited by bradykinin: 0.03 μg (△), and 0.1 μg (○) and acetylcholine: 3 μg (▲) and 10 μg (●). C = controls. C_E = 'Controls', 30 min after start of infusion of 10 ng/ml prostaglandin E_1,-enhanced effect of bradykinin and acetylcholine. R60 = recovery, 60 min after the end of infusion of prostaglandin $F_{2\alpha}$. Each point represents the mean of results from 6 rabbits. Vertical bars represent s.e. mean. $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.005$ (against C_E).

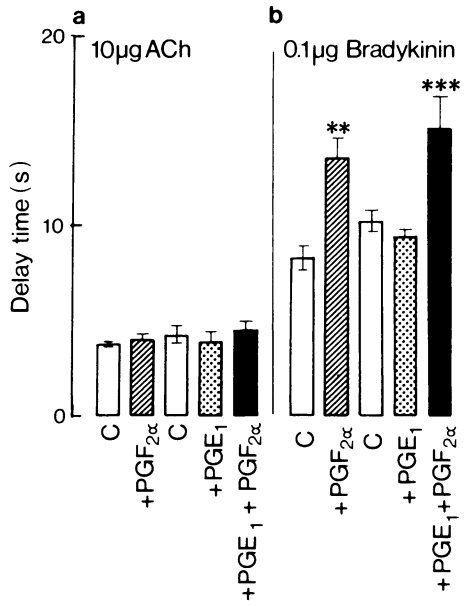

Figure 4 Rabbit ear separated from the systemic circulation. Delay time of reflex fall in blood pressure elited by (a) 10 μg acetylcholine (ACh) and (b) 0.1 μg bradykinin: open columns = controls (C); cross-hatched columns = after infusion of 10 ng/ml prostaglandin $F_{2\alpha}$ for 30–50 min; dotted columns = after infusion of 10 ng/ml prostaglandin E_1 for 20–30 min; solid columns = after infusion of 50 ng/ml prostaglandin $F_{2\alpha}$ concomitantly with 10 ng/ml prostaglandin E_1 for 30–50 min. Each column represents the mean of results from 6 rabbits. Vertical bars show s.e. mean. $^{**}P<0.01$; $^{***}P<0.005$.

response (3 μg instead of 0.3 μg). Under these conditions 0.1 μg prostaglandin $F_{2\alpha}$ no longer reduced the bradykinin response. However, a higher dose (10 μg) significantly ($P<0.01$) enhanced the effect of bradykinin (Figure 5). The effect of acetylcholine was also not influenced by 0.1 μg prostaglandin $F_{2\alpha}$ but was significantly ($P<0.01$) enhanced by 10 μg. Prostaglandin E_1 (0.1 μg) also significantly enhanced the response to bradykinin (Figure 5) and to acetylcholine during indomethacin infusion.

Discussion

Earlier findings showed that E prostaglandins enhanced the algesic effect of bradykinin by a sensitizing action on pain receptors. Such a sensitizing action of E prostaglandins has been found in the human skin (Ferreira, 1972), the rat paw (Willis & Cornelsen, 1973), the spleen (Ferreira, Moncada & Vane, 1973), the knee joint (Ferreira, Moncada & Vane, 1974b; Moncada, Ferreira & Vane, 1974; 1975) and the heart (Vane, 1976) of dogs, and the ear of rabbits (Lembeck & Juan, 1974; Juan & Lembeck, 1974a; 1974b; 1976a; 1976b). Juan & Lembeck (1974b) found on the isolated ear preparation of the rabbit that E prostaglandins enhanced the effect of each of several substances which produced pain following intra-arterial injection, of which the most active were bradykinin and acetylcholine. The effects of these two substances on the rabbit ear preparation differ in the following respects: (a) The algesic effect of bradykinin is strongly attenuated by indomethacin whereas that of acetylcholine is only slightly reduced (Lembeck & Juan, 1974). (b) The prostaglandin antagonist, polyphloretin phosphate, inhibits the algesic effect of bradykin but not that of acetylcholine (Juan & Lembeck, 1976b). (c) Bradykinin in doses eliciting a pain reflex, released some nanograms of E prostaglandins, whereas acetylcholine released barely detectable amounts (Juan & Lembeck, 1976a and c; Lembeck, Popper & Juan, 1976).

Prostaglandin of the E type is also released by bradykinin from the kidney (McGiff, Terragno, Malik & Lonigro, 1972) and spleen (Ferreira, Moncada & Vane, 1973) of dogs, the lung of guinea-pigs (Palmer, Piper & Vane, 1973) and the heart of rabbits

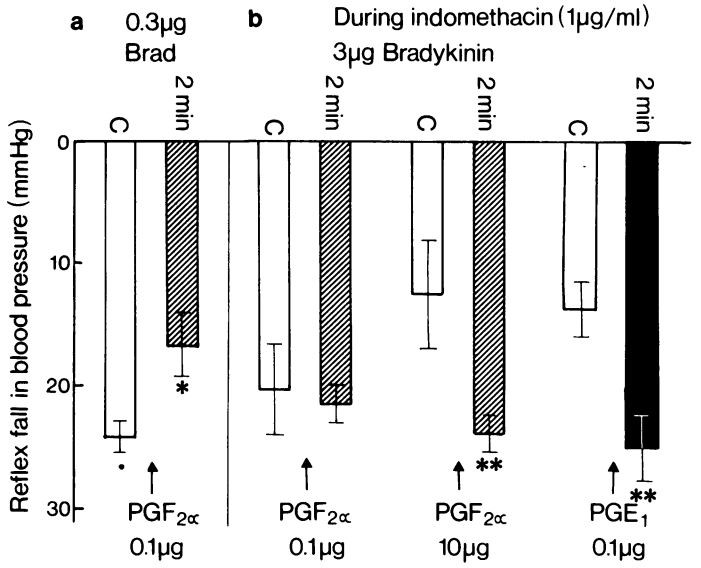

Figure 5 Rabbit ear separated from the systemic circulation. Effect of injection of 0.1 or 10 μg of prostaglandin $F_{2\alpha}$ (cross-hatched columns) and 0.1 μg prostaglandin E_1 (solid column) on reflex fall in mean arterial blood pressure produced by bradykinin (a) 0.3 μg or (b) 3 μg before and more than 30 min after start of infusion of 1 μg/ml indomethacin. C (open columns) = controls; 2 min = bradykinin response 2 min after the prostaglandin. Each column represents the mean of results from 7 rabbits. Vertical bars show s.e. mean. $^*P < 0.05$; $^{**}P < 0.01$.

(Needleman, Marshall & Sobel, 1975). The released E prostaglandins sensitize the pain receptors to the action of bradykinin. Accordingly, indomethacin reduces but does not abolish the algesic effect of bradykinin in dog spleen (Ferreira, Moncada & Vane, 1973) and in the rabbit ear preparation (Juan & Lembeck, 1974a; Lembeck & Juan, 1974) by inhibition of prostaglandin release or synthesis (Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971; Vane, 1971). Polyphloretin phosphate also reduces the effect of bradykinin in the rabbit ear but by interference with the pain enhancing effect of the endogenously released E prostaglandins (Juan & Lembeck, 1976b). Prostaglandin $F_{2\alpha}$, in doses 20 times the dose of prostaglandin E_1, induced incapacitation in the dog knee joint (Rosenthale, Dervinis, Kassarich & Singer, 1972) but did not sensitize pain receptors to bradykinin (Moncada, Ferreira & Vane, 1975). Again, in contrast to prostaglandin E_1, prostaglandin $F_{2\alpha}$ did not elicit abdominal contractions on intraperitoneal injection into mice, indicating lack of nociceptive effect (Collier & Schneider, 1972).

In this study prostaglandin $F_{2\alpha}$ infused intra-arterially into the rabbit isolated perfused ear in very low concentrations (0.1–1.0 ng/ml) reduced the algesic effect of bradykinin but not that of acetylcholine. Surprisingly, a high concentration (10.0 ng/ml) did not further reduce the effect of bradykinin; only slight reductions or slight enhancements were observed. Perhaps prostaglandin $F_{2\alpha}$ in this high concentration has some intrinsic agonist activity on pain receptors. In contrast the effect of acetylcholine was not reduced by any concentration of prostaglandin $F_{2\alpha}$. From these results it could be deduced that the exogenously applied prostaglandin $F_{2\alpha}$ reduced the algesic effect of bradykinin by inhibiting the pain enhancing action of the endogenously released E prostaglandins. In keeping with this, an infusion of prostaglandin $F_{2\alpha}$ reduced the enhancement by prostaglandin E_1 of the algesic effect of both bradykinin and acetylcholine.

The experiments with indomethacin confirm this assumption. During infusion of 1 μg/ml indomethacin the release of prostaglandins by bradykinin from the rabbit ear is abolished (Lembeck, Popper & Juan, 1976). Under these conditions a low dose of prostaglandin $F_{2\alpha}$ no longer reduced the bradykinin effect. However, a high dose of prostaglandin $F_{2\alpha}$ enhanced the effect of bradykinin, clearly showing that it has some intrinsic activity which in Figure 5, is seen to be about one hundredth that of prostaglandin E_1.

The results with low concentrations of prostaglandin $F_{2\alpha}$ in the rabbit ear are similar to those obtained by other authors in other systems. Thus, Brody & Kadowitz (1974) found that prostaglandin $F_{2\alpha}$, which exerted no direct effect on vascular resistance in the perfused dog uterus, markedly

attenuated the vasodilator response to prostaglandin E_1. Both prostaglandins are thought to compete for the same receptors in this system as was also supposed to be so in the uterine artery of dogs (Clark & Brody, 1974) in stomach strips (Splawinski, Nies, Sweetman & Oates, 1973) and in the mouse ovary (Kuehl, Cirillo, Ham & Humes, 1973). A similar interaction seems to exist on or near adrenergic nerve terminals; when neurogenically evoked vasoconstrictor responses were depressed by infusion of prostaglandin E_1, infusion of prostaglandin $F_{2\alpha}$ specifically restored the response to nerve stimulation (Kadowitz, Sweet & Brody, 1971).

Some additional observations on the delay time of the reflex response and the venous outflow were made. These indicate that the different effects of prostaglandins on pain receptors are not secondary to vascular actions. Prostaglandin $F_{2\alpha}$ prolonged the delay time of the reflex response to bradykinin but not to acetylcholine, independent of a simultaneous infusion of prostaglandin E_1. The reason for this prolongation of delay remains unknown. A vascular action which causes a delay of the access of bradykinin to the pain receptors appears to be rather unlikely since prostaglandin $F_{2\alpha}$ did not change the vasoconstrictor effect of bradykinin; it reduced only moderately the vasoconstrictor effect of bradykinin attenuated by prostaglandin E_1. The brief vasodilator effect of acetylcholine was slightly greater in the presence of both prostaglandins than under control conditions.

Prostaglandins have been identified in inflammatory exudates of various origins and have been assumed to play a role in inflammation (see Ferreira, Moncada & Vane, 1974a). The ratio of E and F prostaglandins (E/F) seems to be important for the inflammatory response (Giroud, Velo, Timsit & Willoughby, 1974). We found that prostaglandins of the F series are released in much smaller amounts by bradykinin than are E prostaglandins (Juan & Lembeck, 1976c; Lembeck, Popper & Juan, 1976). But concentrations of as low as 0.1 ng/ml prostaglandin $F_{2\alpha}$ were sufficient to antagonize the pain enhancing action of prostaglandin E_1. Similar concentrations of prostaglandin $F_{2\alpha}$ specifically increased responses to sympathetic nerve stimulation and increased the amount of catecholamines released from the adrenal gland by splanchnic nerve stimulation (Brody & Kadowitz, 1974).

Thus, small amounts of F prostaglandins released by bradykinin or by other stimuli under *in vivo* conditions could occupy prostaglandin binding sites on or near paravascular 'pain receptors' and could antagonize the pain enhancing action of released (or applied) E prostaglandins. In this way interactions of E and F prostaglandins could regulate or modulate algesic actions at the receptor site, the ratio of their concentrations being considered an important factor.

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