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PROSTAGLANDINS RELEASE PLASMA 'RECIPROCAL COUPLING FACTOR' IN ANAESTHETIZED RATS

J.R.S. HOULT & P.K. MOORE[1]

Department of Pharmacology, King's College, Strand, London WC2R 2LS

1 Prostaglandins E_1, E_2, I_2 and endoperoxide analogue U46619 injected intra-arterially (i.a.) into anaesthetized rats at 2 μg/kg caused a substantial increase within 60 min of the plasma activity of prostaglandin 'reciprocal coupling factor' (RCF). RCF is the provisional name for the component(s) of plasma which inhibit microsomal prostaglandin synthesis and enhance cytosolic prostaglandin breakdown.

2 RCF is not released by inactive metabolite 13,14-dihydro-15-keto prostaglandin E_2 (10 μg/kg, i.a.) or acetylcholine or histamine (2 μg/kg, i.a.).

3 We suggest that release by prostaglandins of RCF could provide the basis *in vivo* for a negative feedback mechanism controlling the activity of the prostaglandin system.

Introduction

We found recently that rat and human plasma inhibits microsomal prostaglandin synthesis and stimulates enzymatic inactivation of prostaglandins in cell-free supernatants of organs containing prostaglandin metabolizing enzymes (Moore & Hoult, 1980; Hellewell, Berry, Moore & Hoult, 1980). The plasma component(s) responsible for these *in vitro* effects has not been identified and was given the provisional name of prostaglandin 'reciprocal coupling factor' (RCF). The finding that rat plasma RCF activity was changed after three different pathophysiological treatments in which alterations of the prostaglandin system occur suggested to us that RCF may be a regulator of the prostaglandin system *in vivo*.

We show here that injections in anaesthetized rats of vasoactive prostaglandins themselves cause a rapid increase in plasma RCF activity. However, RCF activity was not increased after injection of histamine, acetylcholine or an inactive prostaglandin metabolite.

Methods

Male Sprague-Dawley rats (200–250 g) were anaesthetized with urethane (650 mg/kg i.p. and s.c.) and the carotid artery cannulated for making injections and removing blood samples.

After preparing the animals and injecting them with 50 units heparin, 0.75 ml blood was removed from the carotid artery to a plastic microcentrifuge tube and centrifuged at 14,000 *g* for 30 s. The resulting plasma was removed and stored at −20°C until assayed. The blood withdrawn was replaced with an equivalent volume of 0.9% w/v NaCl solution (saline). The rats (groups of 6) were then injected with either 0.1 ml saline or 0.1 ml of the same vehicle containing drugs such that the dose administered was 2 μg/kg. After 60 min a further 0.75 ml blood was removed and processed as described. The experiment was then terminated.

The RCF activity of these plasmas was tested in standardized assays of the inhibition of bioassayable prostaglandin synthesis by freshly prepared sheep seminal vesicle microsomes and stimulation of radiolabelled prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) metabolism by freshly prepared 100,000 *g* supernatants of rat caecum, as described before (Moore & Hoult, 1980). That the reduction in formation of bioassayable prostaglandins is a true reflection of the inhibition by plasma of the microsomal conversion of arachidonate to prostaglandin-like products (and not due to a redirection of the pathway towards other non-spasmogenic products) has been shown previously. Thus there is decreased conversion of radiolabelled arachidonate to all products when bovine (Hoult & Moore, 1981) or sheep seminal vesicles are used (Hellewell *et al.*, 1980). Moreover, radioimmunoassay experiments showed that 5% (v/v) rat plasma reduced the formation in 60 min bovine seminal vesicle incubations of PGE_2 from 1091± 56 ng/ml to 174± 9 ng/ml and of 6-keto $PGF_{1\alpha}$ from 214± 18 ng/ml to 120± 13 ng/ml.

Storage at −20°C and freeze/thawing did not affect plasma RCF activity.

[1]Present address: Department of Pharmacology, Chelsea College, Marresa Road, London SW3 3TW

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Results

Intra-arterial injection of PGE_1 2 μg/kg caused an increase in the plasma RCF activity when assayed in blood removed 60 min later. This is shown by both the synthesis-inhibition and metabolism-enhancement assays (Figure 1). The injection of the vehicle did not cause such an increase (curve ▲ in Figure 1). These 'dose-response' curves, obtained by assaying plasma at 0.5, 5.0 and 10.0%, v/v, exhibit a typical upwards inflection as noted previously (Moore & Hoult, 1980) but the reason for this is not known.

Injection of 2 μg/kg of PGE_2 and PGI_2 (prostacyclin) and of the endoperoxide analogue U-46619 (15-S-hydroxy-11α,9α-epoxymethano-prosta-5Z, 13E-dienoic acid, which also has thromboxane A_2-like actions; Malmsten, 1976) also produced similar increases in plasma RCF activity according to both assays.

For brevity, the data for these prostaglandins are not shown graphically but are expressed in terms of the IC_{50} (concentration of plasma as % v/v needed to inhibit microsomal prostaglandin synthesis by 50%) and the AC_{75} (concentration producing 75% activation of $PGF_{2\alpha}$ breakdown in the 100,000 *g* supernatant). Thus PGE_2 caused decreases of the IC_{50} from 3.4% to 0.6% and of the AC_{75} from 2.8% to 1.2%; for PGI_2 the IC_{50} decreased from 7.8% to 0.8% and the AC_{75} from 10.6% to 3.8%; for U46619 the changes were IC_{50} 6.2% down to 1.6% and AC_{75} 7.7% down to 2.7%. These values are all approximate as the 'dose-response' curves are for only 3 points. The first figure in each pair is the control (non-prostaglandin treated) value for the activity of the plasma in the synthesis-inhibition or metabolism-enhancement tests: it should be noted that these values vary between groups because of differences between the RCF activity in plasmas from different groups of rats (the experiments for each prostaglandin were performed at different times using different batches of animals), and because of differences in the biological activities and sensitivities of the freshly prepared assay systems (centrifuged homogenates of sheep seminal vesicle and rat caecum).

13,14-dihydro-15-keto PGE_2 (a biologically inactive metabolite of PGE_2, injected at 10 μg/kg) and acetylcholine and histamine (both 2 μg/kg) did not

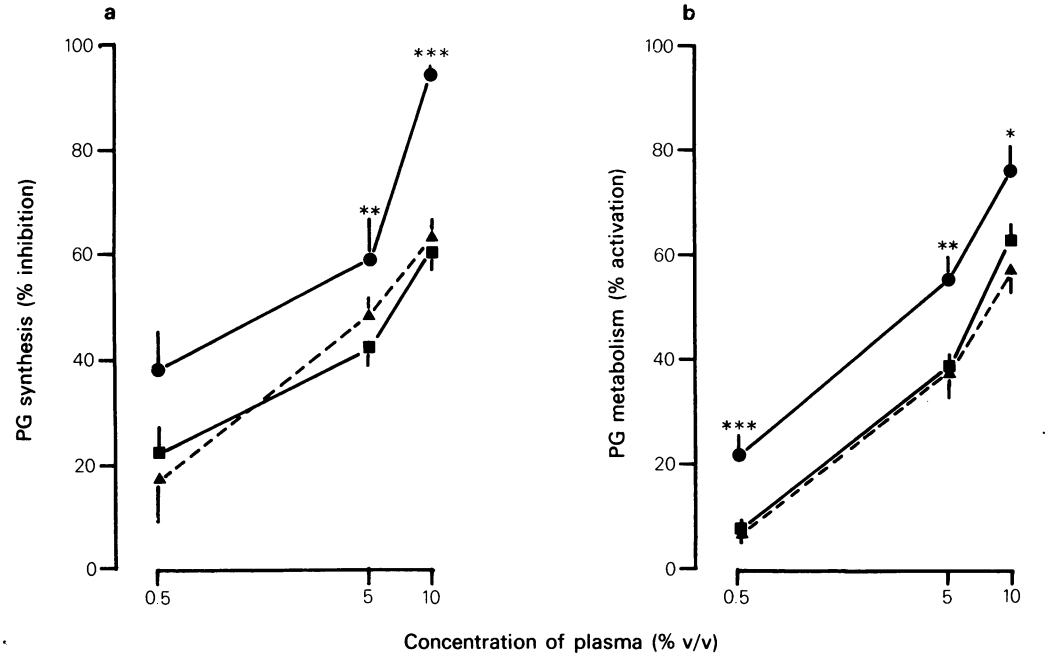

Figure 1 Intra-arterial injection of prostaglandin E_1 (PGE_1, 2 μg/kg, i.a.) into anaesthetized rats increases prostaglandin 'reciprocal coupling factor' activity. (a) Inhibition of prostaglandin (PG) synthesis by sheep seminal vesicle microsomes. (b) Enhancement of $PGF_{2\alpha}$ metabolism by rat caecum supernatant. (■) Plasma taken from rats before injections were made; (▲) plasma taken 60 min after injecting 0.1 ml saline; (●) plasma taken 60 min after injecting 0.1 ml PGE_1 solution. Results show mean values for 10–25 (synthesis) or 6–12 determinations (metabolism) and vertical lines show s.e.mean. Differences significant by Student's unpaired *t* test: $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to pre-injection values.

cause changes in plasma RCF activity according to both assays (though the usual dose-dependent inhibition of synthesis and enhancement of metabolism by plasma were demonstrated). Addition of PGE_1 or PGE_2 to rat plasma *in vitro* (1 μg/ml, a concentration at least 20 times higher than that maximally attainable *in vivo* in these experiments, even assuming no pulmonary degradation) did not alter the ability of plasma to inhibit synthesis or enhance metabolism.

Discussion

These experiments show that prostaglandins E_1, E_2, I_2 and U46619 cause a substantial increase in plasma RCF activity within 60 min of intra-arterial injection in anaesthetized rats. This effect is specific and is not seen after treatment with an inactive metabolite of PGE_2 or with two vasoactive agents given in hypotensive doses. We have also obtained evidence in rats for increased plasma RCF activity following intraperitoneal injection of PGE_1 2 days previously at a dose of 10 μg/kg (Moore & Hoult, unpublished experiments), and after subcutaneous or intra-arterial injection of prednisolone (Moore & Hoult, 1980). A plausible explanation is that these agents stimulate the release (or synthesis and release) of RCF from as yet unidentified cells or organs. Further experiments are required to establish the source, identity and mechanism of release and action of RCF and to determine if its plasma activity can be similarly stimulated in man.

Several interactions between 'plasma factors' and the synthesis of prostaglandins have been identified (see Hoult & Moore, 1981, and Pearson, 1981, for reviews) but it is not yet known to what extent these factors control the activity of the prostaglandin system *in vivo*. Thus effects of plasma, serum or derived components on the synthesis of classical prostaglandins and on platelet thromboxane and vascular prostacyclin formation have all been noted, as well as the interactions of plasma with prostaglandin degradation described in our previous studies.

However, it is of particular interest that as shown here, prostaglandins themselves trigger an increase in plasma RCF activity because this could provide the basis for negative feedback regulatory loop: prostaglandin release is followed by the appearance of a substance(s) which inhibits its further formation and enhances degradation, thus 'switching off' the initiating prostaglandin stimulus.

The validation of such a concept requires that RCF is active *in vivo* or in intact cell systems. Thus far, there is little evidence for this, although in preliminary studies (C.N. Berry & J.R.S. Hoult, unpublished experiments) it was found that addition of rat plasma to the perfusing fluid of the rat isolated lung reduced the synthesis of prostaglandin-like material from arachidonic acid and increased breakdown of radiolabelled $PGF_{2\alpha}$. Further experiments are therefore needed to assess the biological relevance of RCF and of the negative feedback loop proposed in this paper.

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