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BINDING OF PROSTAGLANDIN E_2 TO BLOOD IN MAN

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1 A new dialysis technique has been used to investigate the binding of prostaglandin E_2 (PGE_2) to circulating blood in five subjects.

2 The results indicate that added PGE_2 binds to blood, that the binding is to plasma albumin, and that binding is complete within 40 s of adding PGE_2.

Introduction

The binding of prostaglandins to proteins has been demonstrated *in vitro* in animals (Raz, 1972a; Schaumburg, 1973; Gorman & Miller, 1973; Moore & Wolff, 1973; Litwack, Filler, Rosenfield & Lichtash, 1973; Wakeling & Wyngarden, 1974) and in man (Raz, 1972b,c; Unger, 1972), but there has been no satisfactory method for directly demonstrating whether binding occurs *in vivo*. A modification of the superfusion technique of Gaddum (1953) has been developed (Collier, 1972) which provides a new approach to the investigation of the binding of drugs. We have used this method to study the binding of prostaglandin E_2 (PGE_2) to the blood of man *in vivo*.

Methods

Studies were carried out on patients of either sex aged 27-50 years who received haemodialysis at home for chronic renal failure. Details of the experimental procedure were described to the subjects who gave their informed consent. Permission for the study was given by the Ethical Committee of this hospital.

Subjects were studied in an air-conditioned laboratory in hospital. With the subject resting supine, blood was taken for the estimation of haemoglobin, blood urea and plasma albumin, and dialysis lines were introduced into the subject's shunt or fistula. Coagulation was prevented by the systemic administration of heparin (5,000 iu intravenously as an initial dose, followed by an infusion of 1,000-2,000 iu/h). Blood was then withdrawn from the subject at 50 ml/min and pumped through the central channel of a miniature multipoint dialysis machine (surface area 280 cm^2, blood volume 20 ml) and then returned to the patient. Krebs solution (NaCl 118 mM; KCl 4.7 mM; $CaCl_2$ 2.5 mM; KH_2PO_4 1.2 mM; $MgSO_4$ 1.17 mM; dextrose 5.6 mM; $NaHCO_3$ 25.0 mM; gassed with 95% O_2 + 5% CO_2) was delivered at 5.0 ml/min to the dialysate compartment (volume 20 ml) of the dialysis machine. After passing through the machine, the dialysate superfused a series of isolated assay tissues known to contract in response to PGE_2 (Figure 1). The assay tissues were the rat stomach strip and the chick rectum (Vane, 1957, 1964). Tissue responses to vasoactive substances other than prostaglandins were prevented by pharmacological antagonists added to the superfusing fluid; these were atropine, mepyramine, methysergide, phenoxybenzamine, propranolol and indomethacin. Changes in tissue length were detected using Harvard isotonic transducers (type 386) and recorded on a Watanabe pen recorder (type WTR 281).

PGE_2 (Upjohn Co. Ltd) was infused into the blood before it entered the dialysis machine ('indirect', Figure 1) to give a concentration in blood of 4 or 8 ng/ml. Any PGE_2 free in the plasma was able to pass through the dialysis membrane into the dialysate and contract the superfused assay tissues. The infusion of PGE_2 into blood was maintained until the response of the tissues reached a plateau (Figure 2). The concentration of PGE_2 in the superfusing fluid was estimated by comparing the responses of the assay tissues to the dialysed PGE_2 with those produced by PGE_2 superfused directly over the tissues ('direct', Figure 1). The direct infusion of PGE_2 into the dialysate gave concentrations over the tissues of 0.5, 1.0 and 2.0 ng/ml; each dose

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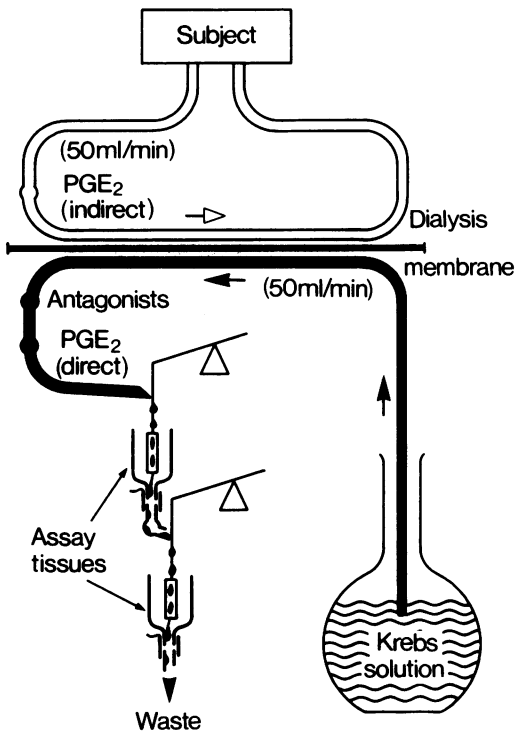

Figure 1 Diagram of the dialysis circuit. Prostaglandin (PGE_2) infused at the point marked 'direct' immediately superfuses the assay tissues. PGE_2 infused at the point marked 'indirect' must cross the dialysis membrane before reaching the tissues.

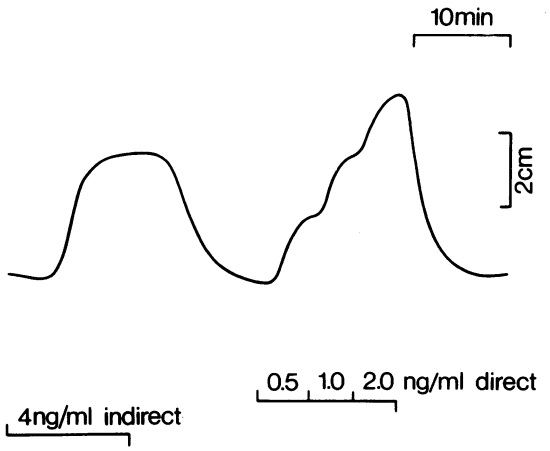

Figure 2 Trace of the response of a rat stomach strip to PGE_2 infused into blood ('indirect') and over the tissues ('direct'). A PGE_2 concentration in blood of 4 ng/ml gives a concentration in the dialysate of 1 ng/ml, a passage of 25% (see text).

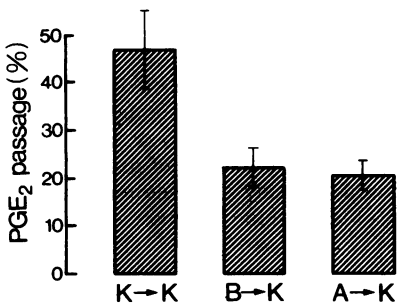

Figure 3 Histogram comparing the passage of PGE_2 across the dialysis membrane from Krebs solution to Krebs solution (K → K, $n = 8$), from blood to Krebs solution (B → K, $n = 8$) and from albumin to Krebs solution (A → K, $n = 7$). The passage from K → K is significantly greater than that from B → K ($P < 0.01$) and from A → K ($P < 0.01$). The vertical lines represent ± 1 s.e. mean.

was maintained until the tissue response began to plateau and the concentration was then doubled to produce a cumulative dose-response curve. The passage of PGE_2 across the membrane was expressed as the percentage concentration of PGE_2 in the dialysate compared to that in blood. For example, when the initial concentration of PGE_2 in blood was 4 ng/ml and that in the dialysate 1 ng/ml (as in Figure 2) the passage was 25%.

After estimating the passage of PGE_2 from blood to Krebs solution, the subject was taken off the dialysis machine and Krebs solution was pumped through the compartment originally occupied by blood. An estimate was then made of the passage of PGE_2 from Krebs solution in the 'blood' compartment to Krebs solution in the dialysate compartment. When this was completed, the 'blood' compartment was perfused with a solution of albumin (2.5-3.75 g/100 ml Krebs solution), and the passage of PGE_2 from albumin to Krebs solution was estimated.

In order to assess whether the passage of PGE_2 was affected by the contact time of PGE_2 with either Krebs solution or albumin before entering the machine, experiments were performed with PGE_2 infused either 40 s upstream from the machine (the delay in the conventional studies) or 120 s upstream. In each study the passage of PGE_2 was estimated first from Krebs solution to Krebs solution and then from albumin to Krebs solution.

Table 1 The age, sex, blood urea and plasma albumin concentration of the five subjects investigated. Subjects 2 and 3 took part in more than one study.

Subject	*Sex*	*Age (years)*	*Blood urea (mg/100 ml)*	*Plasma albumin (g/100 ml)*
1	F	52	51	3.6
2	M	38	76	3.6
			116	4.6
			34	4.0
3	F	51	92	4.8
				4.6
4	F	35	57	4.9
5	M	27	34	5.0
Mean		41	66	4.4

Results

The age and sex of the subjects, together with the concentration of plasma albumin and blood urea, are shown in Table 1. The mean plasma albumin was 4.4 g/100 ml and the mean blood urea 66 mg/100 ml. The raised urea level is consistent with chronic renal failure in patients recently dialysed. The passage of PGE_2 from Krebs solution to Krebs solution, from blood to Krebs solution, and from albumin to Krebs solution is shown in Figure 3. The mean passage of PGE_2 (% ± s.e. mean) into Krebs solution from Krebs solution was 47 ± 8.3 (n = 8), from blood 22 ± 3.3 (n = 8) and from albumin 20 ± 3.4 (n = 7). The difference between the passage of PGE_2 from blood or albumin was not statistically significant, but both were significantly lower than the passage from Krebs solution ($P < 0.01$).

The passage of PGE_2 from Krebs solution to Krebs solution averaged 19% after a 40 s contact time, and 21% after 120 s contact time (n = 2). The passage of PGE_2 from albumin to Krebs solution averaged 13% when the contact time was 40 s, and 14% when the contact time was 120 s (n = 2).

Discussion

The study indicates that PGE_2 binds *in vivo* to human plasma albumin since the passage of PGE_2 from blood to Krebs solution is consistently less than that from Krebs solution to Krebs solution and the passage from blood closely matches the passage from albumin. Binding has not previously been demonstrated *in vivo* in man, although it has been shown to occur *in vitro* in blood samples taken both from animals and man. Binding is probably complete within 40 s of adding PGE_2 since increasing the incubation period of PGE_2 from 40 s to 120 s did not decrease the recovery in the dialysate. That binding occurred within 40 s is consistent with observations *in vitro* that binding of PGE_2 to various tissues reaches a maximum within 1-2 min (Moore & Woolfe, 1973; Schaumburg, 1973).

An alternative explanation for the reduced passage of PGE_2 from blood to Krebs solution as compared to that from Krebs solution to Krebs solution is that erythrocytes might interfere with dialysis by binding or trapping PGE_2. However, PGE_2 does not seem to bind to erythrocytes (Unger, 1972; Greaves & McDonald-Gibson, 1972) and it seems unlikely that trapping is of importance since rapid equilibrium is reached between PGE_2 in erythrocytes and in plasma (Greaves & McDonald-Gibson, 1972). It is also possible that passage of PGE_2 is reduced in the presence of blood because of deposited cells on the membrane. However, in all experiments. passage of PGE_2 from Krebs solution to Krebs solution was measured immediately after the blood study, and the cellular layer would be expected to be still present. The passage of PGE_2 from blood would also appear to be reduced if PGE_2 was rapidly metabolised. In blood, however, PGs are relatively stable (Ferreira & Vane, 1967; Jaffe, Behrman & Parker, 1973).

Blood concentrations of urea and albumin influence binding to plasma proteins. In the

present study blood urea concentrations varied between patients, and in one subject the levels varied more than threefold on different occasions. The urea and albumin concentrations seemed unrelated to binding of PGE_2.

In demonstrating the binding of PGE_2 in man, the present study serves to illustrate the use of a new method for investigating protein binding *in vivo*. The method gives a semi-quantitative estimation of binding and has the advantage over other techniques of avoiding conventional extraction procedures.

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