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THE EFFECTS OF DEPRIVATION OF PROSTAGLANDIN PRECURSORS ON VASCULAR SENSITIVITY TO ANGIOTENSIN II AND ON THE KIDNEY IN THE PREGNANT RABBIT

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1 Pregnant rabbits were deprived of essential fatty acids from day ten of pregnancy, and results compared with a control group on a normal diet.

2 At term, cannulation of jugular and carotid vessels was performed under anaesthesia, to study the vascular sensitivity to angiotensin II and basal blood pressure.

3 Plasma renin levels, urinary electrolytes and protein were measured.

4 Placental and renal tissue was examined histologically.

5 Though no changes were found in tissues, blood or urine, a markedly significant increase in response to angiotensin II was found in the group deprived of essential fatty acids. This parallels the findings in vascular response in human pre-eclampsia.

Introduction

It is now evident that the prostaglandins of the E series (PGEs) play an important role in the control of blood pressure of all mammals studied (Karim, 1976). The mechanisms are incompletely understood, though they appear to act through several systems. The natriuretic and vasodilator actions on the kidneys (McGiff, Crowshaw & Ifskovitz, 1974) and peripheral vasculature (Holmes, Horton & Main, 1963) are probably of greatest importance. In addition to these, there are also relevant actions at adrenal (Dazord, Morera, Bertrand & Saez, 1974) and uteroplacental sites (Speroff, 1975).

It appears that interaction of these prostaglandins with the renin-angiotensin system may be of importance especially in pregnancy. The authors have recently shown such an interaction in rabbits during pregnancy (O'Brien, Filshie & Broughton Pipkin, 1977). In acute experiments prostaglandin E_2, infused into pregnant rabbits, diminished the response to injected angiotensin II (AII). Inhibition of prostaglandin synthesis, by use of indomethacin, produced an increase in basal blood pressure and an increase in the response to injected AII. In hypertensive disease of pregnancy there is an increase in vascular reactivity to exogenous AII (Talledo, Chelsey & Zuspan, 1974). It is reasonable to postulate then, that deficient prostaglandin activity may be related to the increase in vascular sensitivity and thus to the elevation of blood pressure.

To test this hypothesis, rabbits were deprived of prostaglandin precursors (essential fatty acids) during pregnancy and the response to AII was determined. In addition, the other changes characteristic of hypertensive disease of pregnancy were sought.

Methods

New Zealand White rabbits were mated and maintained on a normal diet until day ten of pregnancy. Five of the rabbits were then fed on the diet deficient in essential fatty acids (essential fatty acids $<0.1\%$) (Table 1). The control rabbits were maintained on the normal diet (essential fatty acids $>2.5\%$) (Table 1). Sodium chloride content of both diets was 1%. Water was available *ad libitum*. Both groups continued on their diets until day 28 or 29 at which time they were investigated (term $\sim 30 \pm 1$ day).

Light anaesthesia was induced with intravenous pentobarbitone sodium 30 mg/kg (Nembutal, Abbott Laboratories, Kent). Supplementary doses were given as required and injections were performed when the blood pressure had returned to basal. The trachea was intubated; respiration was spontaneous throughout. The right carotid artery and left external jugular vein were cannulated with PP90 tubing (Portex Ltd., Hythe, Kent). The vagi were preserved. The pulsatile arterial pressure was measured from the cannula in

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continuity with the carotid artery, by means of a Bell and Howell 4-327-L22 transducer. Synthetic angiotensin II (α-1-Asp^1-Val^5 angiotensin II amide (Hypertensin, Ciba Ltd., Horsham, Surrey)) was made up to 40 μg/ml in distilled water and stored in aliquots at $-25°C$. Appropriate dilutions were made for each experiment with 0.9% w/v NaCl solution (saline) such that 30 ng/kg of AII could be administered in 0.5 ml of solution. This dose was shown in the previous studies, to give a transient increase in blood pressure in the region of 20% (O'Brien *et al.*, 1977).

Basal blood pressure was measured for 10 min after cannulation. The vascular sensitivity, defined as the increase in diastolic blood pressure in response to the injected AII, was determined by administration of 30 ng/kg of the solution via the jugular cannula. Three injections were given to each rabbit, as well as control injections of saline, in random order.

Arterial blood was then taken for estimation of plasma renin activity (PRA) and plasma renin concentration (PRC). These were measured by radioimmunoassay using a modification of the method of Haber, Koerner, Page, Klinman & Purnode (1969). Renin substrate, obtained from sheep six days postnephrectomy, was used in the plasma renin concentration assay according to the method of Skinner (1967). Following this blood sampling, the animal was killed with an overdose of Nembutal.

Urine was obtained by direct needle aspiration from the bladder. Urinary protein was measured by the sulpho-salicylic acid precipitation technique and photometric analysis at 610 nanometers. Urinary electrolytes were determined by flame photometry.

A placenta and a kidney were removed and placed in formalin. Both renal and placental tissue were examined histologically after staining with haematoxylin/eosin stain and M.S.B. (Martius, Scarlet, Blue) for fibrin.

Results were analysed for statistical significance by Student's t test where data were of similar variance. Where an F test showed significant difference in variance a modification of the t test was used (Bailey, 1964).

Table 1 Calculated analysis of the experimental and control diets

	Control diet	*Experimental diet*
Protein	16.5%	19.0%
Essential fatty acids	2.5%	<0.1
Carbohydrate	37.6%	46.0%
Fibre	10.3%	14.0%
Sodium chloride	1.0%	1.0%

All other components of the diet are identical as shown by calculated analysis.

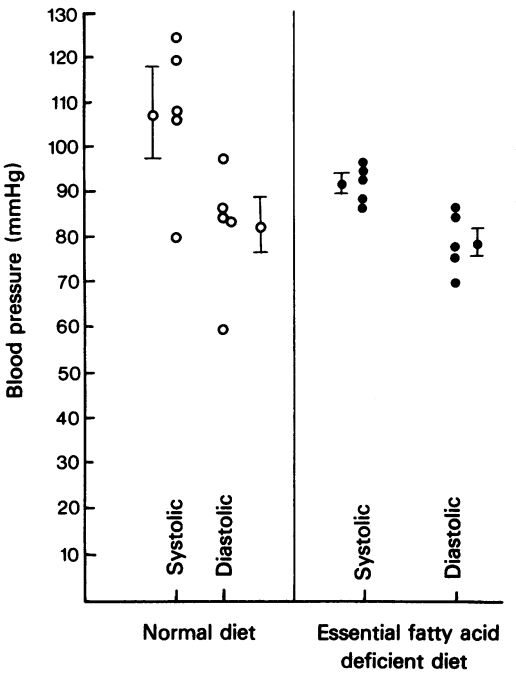

Figure 1 Systolic and diastolic blood pressure in the two groups of rabbits. Results are expressed in mmHg. Mean values are given; vertical bars show s.e. mean.

Results

Blood pressure

Deprivation of essential fatty acids did not give rise to a significant change of the basal blood pressure (Figure 1, Table 2). The systolic blood pressure of the experimental group appeared slightly diminished though this was not statistically significant.

Vascular sensitivity

The increase in diastolic blood pressure in response to 30 ng/kg of injected AII was significantly greater in the group deprived of essential fatty acids ($P < 0.005$) than in controls (Figure 2, Table 2).

Renin

Plasma renin concentration appeared to be unaltered by the deprivation of essential fatty acids (Figure 3a). Plasma renin activity and the ratio of plasma renin activity to plasma renin concentration (PRA/PRC) both appeared depressed in the experimental group

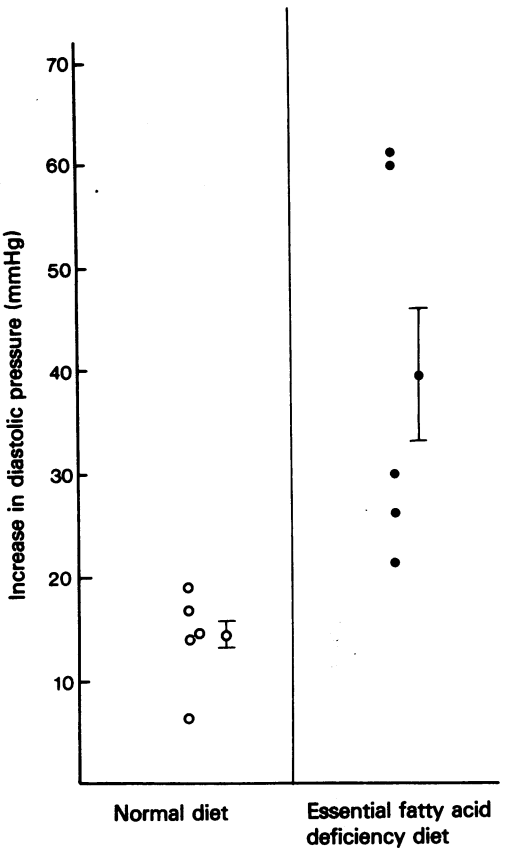

Figure 2 Increase in diastolic pressure (mean of three observations) in response to 30 ng/kg angiotensin II. Mean values are shown; vertical bars show s.e. mean. The difference in response was highly significant ($P < 0.005$).

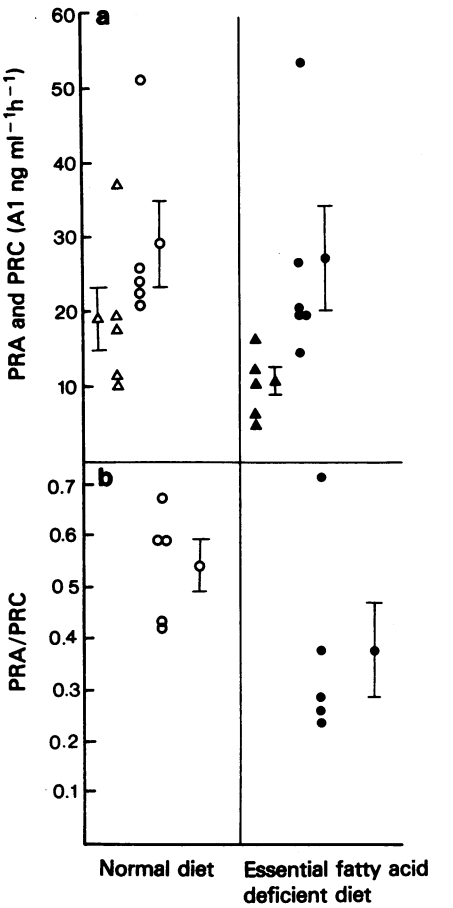

Figure 3 (a) Plasma renin activity (PRA, △▲) and concentration (PRC, ○●) and (b) PRA/PRC ratio in the two groups of rabbits. Mean values are shown, vertical bars indicate s.e. mean.

(Figure 3a,b), although statistical significance was not shown.

Urine

Proteinuria was found in both groups of rabbits (Table 2). It was not elevated in the experimental group. Sodium/potassium ratio was similar in both groups (Table 2).

Histology

There were no significant histological changes in the placental or renal tissue of either group. The only detectable change was that of fibrin deposition in some glomeruli of one rabbit. The same rabbit also showed the greatest increase in vascular sensitivity. None of the other changes associated with human pre-eclampsia was demonstrated in these tissues.

Discussion

Since we had previously shown that the acute inhibition of prostaglandin synthesis caused an elevation of the basal blood pressure of pregnant rabbits (O'Brien *et al.*, 1977), we were surprised to find that the deprivation of prostaglandin precursors failed to produce a similar change. However, the diminution of prostaglandin activity presumed to have occurred was of substantially longer duration in the present experiments. Thus, it is feasible that long term alterations of vascular tone had been compensated for by renal or adrenal changes. Hypertension has only pre-

Table 2 Mean (± s.e.) values for blood pressure, vascular sensitivity, renin, urinary electrolytes and protein in rabbits receiving normal diet and rabbits receiving diet deficient in essential fatty acids

	Blood pressure	*Vascular sensitivity* (a) *% increase* (b) *Absolute increase in diastolic BP*	*Plasma renin activity (PRA)* (AI ng ml^{-1} h^{-1})	*Plasma renin concentration (PRC)* (AI ng ml^{-1} h^{-1})	$\frac{PRA}{PRC}$	*Urinary protein* (g/l)	*Urinary sodium Potassium*	*Histological changes*
Normal diet	*108.0 ± 10.1* 83.0 ± 6.2	(a) 17.9 ± 2.3 (b) 14.2 ± 1.9	16.2 ± 4.0	29.0 ± 5.5	0.54 ± 0.05	0.46 ± 0.2	0.59 ± 0.5	None
Essential fatty acid deficient diet	*92.0 ± 1.6* 79.0 ± 3.0	(a) 51.3 ± 6.8 (b) 39.6 ± 7.0	9.2 ± 1.7	27.4 ± 6.9	0.37 ± 0.09	0.21 ± 0.2	0.8 ± 0.26	Min. fibrin deposition in glomeruli of one animal
Statistical significance	$P < 0.2$ $P < 0.3$	(a) $P < 0.005$ (b) $P < 0.005$	$P < 0.2$	$P < 0.9$	$P < 0.2$	$P < 0.5$	$P < 0.8$	

viously been produced by long term interference with prostaglandin synthesis, when this was superimposed on other changes, such as dietary salt loading (Rosenthal, Simone & Silbergleit, 1974). We had suspected that the alteration of prostaglandin synthesis in addition to the existing unstable water and electrolyte balance of pregnancy might have been sufficient to induce hypertension. We were also unable to show significant changes in the degree of proteinuria or alterations in electrolyte balance. None of the histological changes of pre-eclampsia was seen in renal or placental tissue.

However, we were able to show a very significant increase in the vascular sensitivity (i.e. the response to injected angiotensin II) in the group deprived of essential fatty acids ($P < 0.005$). This was even greater than had been expected in the light of our previous acute experiments.

It is arguable that the kidney may be involved through changes in renin production. Larsson, Weber & Anggard (1974) showed that acute inhibition of prostaglandin synthesis, by indomethacin, lowered plasma renin activity in rabbits. AII levels might be expected to fall proportionately, resulting in an enhanced response to exogenous AII. The slightly lowered PRA levels in our experimental group may also have been a response to lower circulating prostaglandin levels. However, the difference in PRA/PRC ratio suggests that plasma renin substrate changes occurred, rather than alterations in renin production. Secondly, the rabbits showing the greatest increase in vascular sensitivity had the highest PRA levels.

The deprivation of essential fatty acids has been postulated to give rise to deficient prostaglandin activity (Rosenthal *et al.*, 1974). This, in turn, may lead to an imbalance in the AII/prostaglandin interaction at peripheral vascular sites. This could reduce any protective function of the prostaglandins in response to exogenous angiotensin II, resulting in the observed increase in the vascular sensitivity. However, we have not produced direct evidence that there is diminution in prostaglandins at peripheral vascular sites, and circulating prostaglandin levels would provide little additional information. It is possible, then, that the alteration of response to angiotensin II is produced via a mechanism other than diminution in prostaglandin activity.

Stamler (1959) induced a fat-soluble vitamin deficiency in rats, producing a pre-eclampsia like state; a similar pathway may be involved here.

There is also evidence that in essential fatty acid deficient rats, *in vitro* conversion of arachidonic acid to PGE2 is increased in the renal medulla compared to controls (Kaa, 1976). This may suggest that excess prostaglandin activity may be the mechanism involved or, conversely the increase in natriuretic renal prostaglandins may account for the normal blood pressure in the presence of increased vascular sensitivity in our experiments.

Arachadonic acid is also converted to thromboxane and prostacyclin compounds, deficiency of which may lead to alteration in vascular sensitivity. These compounds have not been fully investigated in this context, but should be considered as a possible mechanism.

It seems probable, though these investigations are not conclusive, that the prostaglandin pathway is closely linked to the development of altered vascular sensitivity. Speroff (1975) has previously postulated that the prostaglandins exert a protective effect in human pregnancy, counteracting the potential vasoconstrictor effect of the raised AII levels in normal pregnancy (Skinner, Lumbers & Symonds, 1972) and hypertensive pregnancy (Symonds, Broughton Pipkin & Graten, 1975). Vascular reactivity to exogenous AII is indeed significantly reduced in the normotensive pregnant woman in whom peripheral venous levels of PGE have recently been found to be four-fold compared with those in non-pregnant women (Ferris, Venuto & Bay, 1976). Such reactivity is increased in hypertensive pregnancy (Talledo *et al.*, 1974). Peripheral venous PGE levels have not been measured in this condition.

In summary, the results of our study show that the deprivation of essential fatty acids in pregnancy results in a marked increase in the pressor response to injected AII. It is therefore, not unreasonable to suggest that a diminution in prostaglandin activity, however invoked, may allow a greater degree of responsiveness to the vasoconstrictor action of the high circulatory levels of AII. Other mechanisms involving fatty acids must also be considered, though further work will be needed to confirm or refute the role of prostaglandins in the alteration of vascular sensitivity and possibly hypertensive disease of pregnancy.

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