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PROSTAGLANDIN SYNTHESIS AND RENAL FUNCTION IN MAN

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SUMMARY

1. Experiments were performed to determine the changes in renal function which occur following prostaglandin synthetase inhibition in healthy conscious humans. It was hoped that such experiments could provide information on the mechanism by which renal prostaglandin synthesis influences urinary excretion.

2. In water-diuretic male subjects (receiving a slow saline infusion) the renal excretion of sodium and water was reduced following I.V. acetylsalicylic acid (1 g) administration, while the effective renal plasma flow (*p*-aminohippurate clearance), and glomerular filtration rate (inulin clearance) remained unaltered.

3. In normally hydrated female subjects on an unrestricted diet, the mean urinary prostaglandin E output was 8·5 ng/hr. The renal excretion of sodium, water and urinary prostaglandin E were significantly reduced ($P < 0·05$) following oral acetylsalicylic acid (1·2 g) administration.

4. In normally hydrated female subjects on an unrestricted diet the renal excretion of sodium and water was reduced following oral paracetamol (1·5 g) administration.

5. It is concluded that following renal prostaglandin synthetase inhibition in conscious humans, the excretion of sodium and water can be reduced without measurable changes in the glomerular filtration rate or effective renal plasma flow. It is suggested that in conscious healthy humans, the kidney may continually synthesize prostaglandin which might help to maintain sodium and water excretion by a direct action on the renal tubule without influencing renal blood flow. The relevance of this hypothesis to the intrarenal location of prostaglandin synthetase is discussed.

INTRODUCTION

Evidence obtained from the dog, rabbit and rat suggest that prostaglandins synthesized in the kidney may be involved in the regulation of renal blood flow and urinary excretion (Anderson, Berl, McDonald & Schrier, 1976; McGiff & Malik, 1976). In man, prostaglandins of the E series and the F series have been isolated from renal medullary tissue (Spector, Zusman, Caldwell & Speroff, 1974), renal venous blood (Papanicolaou, Safar, Hornych, Fontaliran, Weiss, Bariety & Milliez, 1975) and urine (Frolich, Sweetman, Carr, Splawinski, Watson, Anggard & Oates, 1973). The cortex

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of the human kidney also contains prostaglandins of the E and F series, although in smaller quantities than the medulla (Spector *et al.* 1974); a similar finding has been demonstrated in the rabbit (Larsson & Anggard, 1976). Recently, prostacyclin (PGI_2 has been shown to be a major prostaglandin in the renal cortex of the rabbit (Whorton, Smigel, Oates & Frolich, 1978) and prostacyclin-like activity has also been demonstrated in human renal cortical tissue (Remuzzi, Cavenaghi, Mecca, Donati & de Gaetano, 1978). The ability of the human kidney to synthesize prostaglandin has generally been assessed from the urinary output of PGE since the urinary content of PGE in women is thought to be derived solely from the kidney (Frolich, Wilson, Sweetman, Smigel, Nies, Carr, Watson & Oates, 1975).

Present evidence indicates that the major effects of prostaglandins on renal function in man, as in several other species, include vasodilation and natriuresis. However the evidence for such effects which has been obtained from human subjects is of a less direct nature than that obtained using experimental animals. Little information exists on the changes in human renal function induced by PGE_2 the major renal prostaglandin, but prostaglandins of the A series have been shown to increase sodium output in healthy subjects (Fichman, Littenburg, Brooker & Horton, 1972) and to increase sodium output together with renal blood flow in hypertensive patients (Carr, 1970). The antinatriuretic and fluid retaining properties of anti-inflammatory drugs (Hanzlik, Scott & Reycraft, 1917; Bachman, Calkins & Bauer, 1963; Meiers & Wetzels, 1964), which have the ability to reduce prostaglandin synthesis (Flower, 1974) are consistent with prostaglandins being diuretic and natriuretic in man, as is the ability of prostaglandin synthetase inhibitors to potentiate the action of antidiuretic hormone (Berl, Raz, Wald, Horowitz & Czaczkes, 1977) and to inhibit diuretic agents such as spironolactone (Tweedale & Ogilvie, 1973) and frusemide (Patak, Mookerjee, Bentzel, Hysert, Babej & Lee, 1975). In conditions where the urinary output of PGE is elevated, e.g. Bartter's Syndrome (where renal prostaglandin synthesis may be high) the excessive fluid and sodium loss which occurs can be successfully treated with a prostaglandin synthetase inhibitor such as indomethacin (Gill, Frolich, Bowden, Taylor, Keiser, Seyberth, Oates & Bartter, 1976).

Several mechanisms have been suggested to account for the natriuretic and diuretic properties of prostaglandins, including an increase in total renal blood flow, and its distribution to the medulla (McGiff & Malik, 1976), antagonism of the action of antidiuretic hormone (Berl *et al.* 1977), or a more direct action on the renal tubule (Roman & Kauker, 1978; Haylor & Lote, 1980).

In the present investigation three experiments were performed to evaluate possible mechanisms by which prostaglandin production might influence urinary excretion in healthy conscious humans. In the first experiment, urinary excertion and renal haemodynamics were monitored in male subjects before and after administration of the prostaglandin synthetase inhibitor, acetylsalicylic acid. In the second experiment, renal prostaglandin synthesis, assessed from measurements of urinary prostaglandin E (PGE) output, and urinary excretion were monitored in female subjects. Finally, in the third experiment urinary excretion was measured in female subjects before and after administration of another prostaglandin synthetase inhibitor, paracetamol.

METHODS

1. *Measurement of urinary excretion together with the glomerular filtration rate and effective renal plasma flow; effect of acetylsalicylic acid*

The renal clearances of inulin and *p*-aminohippuric acid, which are equivalent to the glomerular filtration rate and the effective renal plasma flow respectively (Smith, 1951). were measured in healthy conscious male subjects undergoing a water diuresis. Accurate measurements of renal clearance, without bladder catheterization, can only be made in male subjects with a high urine flow. On the experimental day, male subjects (aged 18–25 years), who had not taken any drugs during the previous 2 weeks, were asked to refrain from eating breakfast. Starting at 8 a.m. subjects drank 500 ml. water every 15 min for 1 hr followed by 150 ml. every 15 min for the remainder of the experiment. During the first 2 hr period subjects practised complete bladder emptying and thereafter produced 30 min urine collections. At 10 a.m. a polypropylene cannula was placed in the left subclavian vein and a loading dose of inulin 2 g (Kerfoot) and sodium *p*-aminohippurate 2 g (Merck, Sharp and Dome) was administered by slow intravenous injection, followed by a maintenance infusion of inulin (15·5 mg/ml.) and *p*-aminohippurate (12 mg/ml.) in 0·153 M-sodium chloride, delivered at 1 ml./min. The infusion was contained in a collapsible plastic bag and delivered using a peristaltic pump (Watson Marlow RHE 22 modified). The right subclavian vein was cannulated for the collection of blood samples and, following a 2 hr equilibration period, blood (2 ml.) and urine were collected every 30 min for a 6 hr period. Blood samples were immediately centrifuged and the plasma was removed. The inulin, *p*-aminohippurate, sodium contents and osmolality of plasma and urine were assayed (see chemical analyses). For each 1 hr period the renal clearances of inulin and *p*-aminohippurate were calculated from their respective plasma concentrations and urinary outputs.

At 3 p.m., 1 g acetylsalicylic acid (meglumine salt) was administered by slow intravenous injection. The injection was freshly prepared in the Pharmacy Department, Queen Elizabeth Hospital, Birmingham. It consisted of a 5% solution of acetylsalicylic acid B.P. and meglumine B.P. (May and Baker) sterilized by filtration through a 0·22 μm filter.

2. *Measurement of urinary excretion together with urinary prostaglandin E output; effect of acetylsalicylic acid*

Six healthy female subjects (aged 18–45 years) were asked to produce three consecutive 3 hr urine samples on each of two days while carrying out their normal working routine. Subjects were requested to undergo a similar activity and diet on the night before and during the first (control) and second (experimental) day. Measurements of urinary prostaglandin E output can be used to assess renal prostaglandin production only in female subjects, because such a correlation is possible when prostaglandin E content of urine is derived solely from the kidney (Frolich *et al.* 1975), and in male subjects prostaglandin in urine may also be derived from the accessory sex glands. All subjects were requested to refrain from sexual activity during the previous night to avoid possible contamination from seminal prostaglandins. Each subject received a placebo (anhydrous citric acid 120 mg; calcium carbonate 120 mg; sodium saccharin 12 mg) at the end of the first 3 hr period on the control day, and 4 × 300 mg Soluble Aspirin Tablets B.P. at the same time on the experimental day. Fluid intake was 120 ml./hr. Urine samples were assayed for sodium, osmolality and PGE (see chemical analyses).

3. *Measurement of urinary excretion; effect of paracetamol*

Six healthy female subjects were asked to collect three complete 3 hr urine samples on each of two days. Fluid intake was 60 ml./hr for the period of urine collection. At the end of the first 3 hr period on the second day 3 × 500 mg Paracetamol Tablets B.P. were given; no placebo was administered on the first day. The sodium content and osmolality of each urine sample was measured (see chemical analyses).

4. Chemical analysis

(i) *Inulin and p-aminohippurate assay.* Plasma and urine samples were subjected to protein precipitation (Somogyi, 1930). Inulin was assayed by the method of Bojesen (1952) and *p*-aminohippurate by the method of Smith, Finkelstein, Aliminosa, Crawford & Graber (1945).

(ii) *Sodium and osmolality.* Osmolality was measured using a freezing-point depression osmometer (Knauer) and sodium was assayed by flame photometry.

(iii) *Prostaglandin E content of urine.* Samples were subjected to concentration, extraction, column chromatography and bioassay using techniques developed from those employed by Vane (1957), Caldwell, Brock, Gordon & Speroff (1972), Frolich *et al.* (1973) and Sirois & Gagnon (1974).

Twenty nCi tritiated prostaglandin E_2 [^{3}H]PGE_2 (Radiochemical Centre, Amersham) was added to each urine sample immediately after collection. Urine was concentrated (to 30–50 ml.) by rotary evaporation at 35 °C and the sediment removed by centrifugation. The concentrated sample was adjusted to pH 3 with hydrochloric acid (Bygdeman & Samuelsson, 1966) and extracted with 3 × 100 ml. chloroform (A. R. redistilled). The chloroform extract was washed with hydrochloric acid (pH 3), rotary evaporated (to 3–5 ml.) at 35 °C, then evaporated to dryness under nitrogen and stored at − 20 °C.

Extracts were redissolved in solvent 1 (see below) and added to activated silicic acid/celite columns (2:1 by weight; 0·6 × 6 cm). The columns were eluted under pressure at 1 ml./min with 90 ml. solvent 2, followed by 24 ml. solvent 3. The fraction eluted by solvent 3 was rotary evaporated (to 3–5 ml.) at 35 °C and evaporated to dryness under nitrogen.

Chromatographed residues were bioassayed on stomach fundic strips taken from Sprague-Dawley rats (250–350 g). The fundic strips were incubated at 37 °C in Tyrode's solution which also contained the following blocking drugs: propranolol (ICI) 3 μg/ml., phentolamine (CIBA) 0·1 μg/ml., mepyramine (May and Baker) 0·1 μg/ml., atropine (Sigma) 0·1 μg/ml. and indomethacin (Merck, Sharp and Dome), 4 μg/ml., in a 2 ml. organ bath. A constant tension of 1 g was applied to the tissue and tension was recorded using a smooth muscle transducer (Statham 16 oz.) and chart recorder (Devices M4). Residues were redissolved in Tyrode solution (with blockers) and bracket assayed against standard concentrations of PGE_2 (Upjohn). Contractile activity present in residues could be inhibited with the prostaglandin antagonist SC-19920 (Searle) (Sanner, 1972). If the tissue failed to respond to urine residues the sensitivity of the tissue to prostaglandin was tested with standard concentrations of PGE_2.

An aliquot of each urine residue, prepared for bioassay, was assayed for tritium (Nuclear Chicago Scintillation Counter). The activity recovered was compared to the activity of the quantity of [^{3}H]PGE_2 added to each urine sample. The bioassay results were expressed as PGE-like activity in terms of PGE_2, adjusted for recovery.

Chemicals. Chromatography solvents (all A. R. grade): solvent 1, benzene:ethyl acetate:methanol (6:4:1, v/v); solvent 2, benzene:ethyl acetate (6:4, v/v); solvent 3, benzene:ethyl acetate:methanol (60:40:4, v/v).

Permission to perform the above studies in human subjects was granted by the Research Ethical Committee, Queen Elizabeth Hospital, Birmingham.

RESULTS

Experiment 1. Renal function before and after acetylsalicylic acid in water-diuretic male subjects

Fig. 1 shows the effect of intravenous acetylsalicylic acid (1 g), given as the meglumine salt, on urinary excretion measured at 30 min intervals in water-diuretic male subjects. The urine flow, sodium output and free-water clearance were all significantly reduced ($P < 0·01$) in the 60 min period following acetylsalicylic acid administration, while the urine osmolality increased ($P < 0·05$). Three hr after acetylsalicylic acid treatment all measurements of urinary excretion had returned to their pre-treatment values. In Table 1 measurements of the glomerular filtration rate and

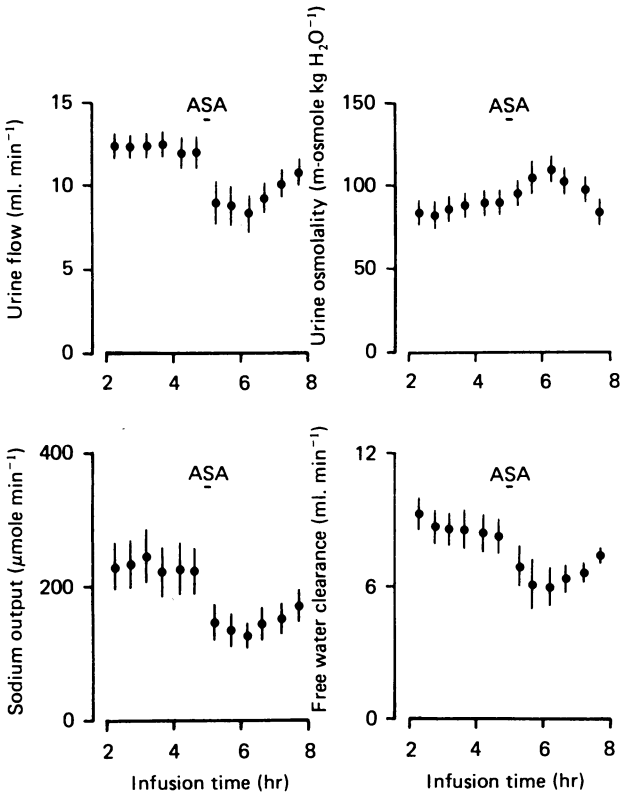

Fig. 1. Urinary excretion in water-diuretic male subjects receiving an isotonic infusion (saline containing inulin and PAH; 1 ml./min) before and after intravenous acetylsalicylic acid (1 g) (ASA) given as the meglumine salt. The infusion time denotes the time from the start of the isotonic infusion. Vertical bars represent S.E. of the mean (n = 6).

TABLE 1. Renal function in water-diuretic male subjects receiving a slow isotonic infusion (saline containing inulin and PAH; 1 ml./min) before and after intravenous acetylsalicylic acid (1 g) given as the meglumine salt. The infusion time denotes the time from the start of the isotonic infusion. All values are expressed as the mean ± S.E. of mean (n = 6). P value (paired t test) compares measurement of renal function before and after acetylsalicylic acid. * $P < 0.05$, ** $P < 0.01$.

	Infusion time (hr)	
Renal function measurement	3–5	5–7, Aspirin 1 g after 5 hr
Urine flow (ml. min^{-1})	12·25 ± 0·90	9·41 ± 1·08**
Osmolality (m-osmole kg H_2O^{-1})	89·5 ± 6·3	105·9 ± 7·8*
Osmolal output (m-osmole min^{-1})	1·07 ± 0·091	0·92 ± 0·117**
Sodium output (μmole min^{-1})	228 ± 40	139 ± 23**
Free water clearance (ml. min^{-1})	8·41 ± 0·87	6·33 ± 0·95**
Glomerular filtration rate (ml. min^{-1}) (inulin clearance)	121·3 ± 6·1	120·9 ± 6·2
Effective renal plasma flow (ml. min^{-1}) (PAH clearance)	595 ± 63	597 ± 56

effective renal plasma flow (clearances of inulin and *p*-aminohippurate respectively) and urinary excretion taken over the 2 hr period following acetylsalicylic acid are compared to the values obtained during the previous 2 hr. The reduction in urinary excretion which occurred following acetylsalicylic acid was unaccompanied by measurable changes in either inulin or *p*-aminohippurate clearance.

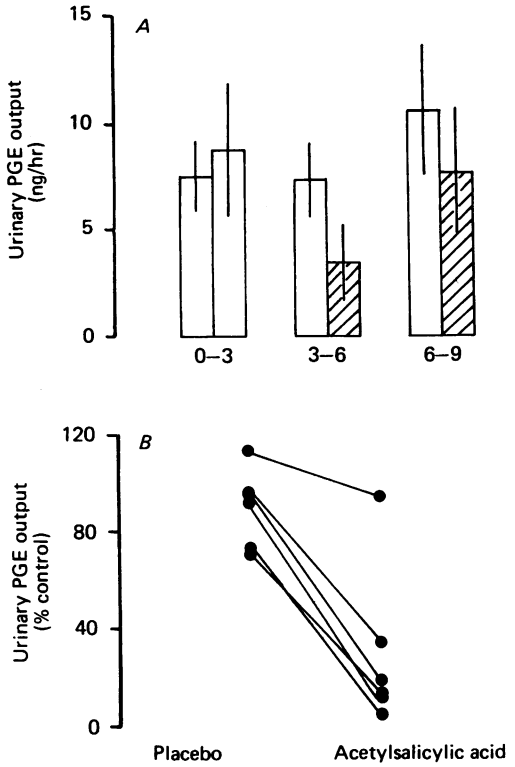

Fig. 2. Urinary prostaglandin E output in normally hydrated female subjects before and after oral acetylsalicylic acid (1·2 g) administration. In *A*, paired columns indicate urinary PGE output (expressed as PGE_2-like activity) measured over three consecutive 3 hr periods on each of two days. After the first 3 hr period (0–3) subjects received a placebo (left-hand columns) or acetylsalicylic acid (right-hand columns). Columns denoting urinary PGE output following acetylsalicylic acid are hatched. In *B*, for each subject the urinary PGE output in the 3 hr period after either the placebo or acetylsalicylic acid was expressed as a percentage of the value obtained in the previous 3 hr.

Experiment 2. Urinary prostaglandin E output before and after acetylsalicylic acid together with measurements of urinary excretion

Prostaglandin E was detected in each of four 3 hr urine samples collected from six healthy normally hydrated female subjects. The mean urinary PGE output, $8{\cdot}5 \pm 1{\cdot}8$ ng/hr ($n = 24$) was at the lower end of previously published values which however were measured mainly from 24 hr urine collection (Frolich *et al.* 1975; Tan, Sweet & Mulrow, 1978). The urinary prostaglandin E outputs in three consecutive 3 hr periods taken on each of two days are shown in Fig. 2*A*. The excretion of urinary PGE was significantly ($P < 0{\cdot}05$) reduced following oral acetylsalicylic acid (1·2 g).

This effect is demonstrated more dramatically in Fig. 2*B* where for each subject the urinary PGE output during the 3 hr period following treatment with either placebo or acetylsalicylic acid was expressed as a percentage of the value obtained in the previous 3 hr. In five out of six subjects the urinary PGE output was markedly reduced following acetylsalicylic acid. The one subject who did not respond had a high

TABLE 2. Urinary excretion in normally hydrated female subjects before and after acetylsalicylic acid. Results are expressed as mean ± S.E. of mean (n = 6) from three consecutive 3 hr urine samples collected on each of two separate days. At the end of the first 3 hr period (0–3) on the first day (C) a placebo was given while oral acetylsalicylic acid (1·2 g) was administered at the same time on the second day (A). The P value (non-paired t test) compares measurement of urinary excretion after placebo or acetylsalicylic acid. * $P < 0{\cdot}01$

		Time (hr)		
Urinary excretion		0–3	3–6	6–9
Urine flow (ml. min^{-1})	C	0·92 ± 0·27	1·45 ± 0·28	1·50 ± 0·35
	A	1·35 ± 0·36	0·76 ± 0·10*	0·96 ± 0·19
Osmolality (m-osmole kg H_2O^{-1})	C	700 ± 88	490 ± 79	493 ± 125
	A	546 ± 103	502 ± 66	456 ± 60
Sodium output (μmole min^{-1})	C	70·8 ± 18	67·5 ± 13	72·0 ± 16
	A	94·0 ± 16	24·5 ± 5·1*	60·3 ± 12·2
Osmolal output (m-osmole min^{-1})	C	0·55 ± 0·067	0·65 ± 0·206	0·58 ± 0·061
	A	0·56 ± 0·074	0·36 ± 0·037*	0·48 ± 0·066

TABLE 3. Urinary excretion in normally hydrated female subjects before and after paracetamol administration. Results are expressed as mean ± S.E. of mean (n = 6) from three consecutive 3 hr urine samples collected on each of two separate days. At the end of the first 3 hr period on the first day (C) no treatment was given while oral paracetamol (1·5 g) was administered at the same time on the second day (P). The P value (non-paired t test) compares measurement of urinary excretion after paracetamol with value at same time on the control day. * $P < 0{\cdot}02$

		Time (hr)		
Urinary excretion		0–3	3–6	6–9
Urine flow (ml. min^{-1})	C	0·86 ± 0·33	0·69 ± 0·16	0·82 ± 0·26
	P	0·93 ± 0·13	0·41 ± 0·03*	0·55 ± 0·04
Osmolality (m-osmole kg H_2O^{-1})	C	797 ± 98	803 ± 87	764 ± 98
	P	690 ± 85	882 ± 85	825 ± 43
Sodium output (μmole min^{-1})	C	120 ± 40	82 ± 18	89 ± 25
	P	100 ± 11	39 ± 2*	61 ± 6
Osmolal output (m-osmole min^{-1})	C	0·57 ± 0·13	0·51 ± 0·08	0·53 ± 0·12
	P	0·60 ± 0·03	0·35 ± 0·02*	0·45 ± 0·04

basal output of PGE. Measurements of urinary excretion which were taken at the same time as urinary PGE output are shown in Table 2. Following acetylsalicylic acid treatment there was a reduction in the urine flow, osmolal output and sodium output as in Expt. 1; there was however, no measurable change in urine osmolality.

Experiment 3. Urinary excretion before and after paracetamol

The urine flow, osmolal output and sodium output were reduced ($P < 0{\cdot}02$) in six healthy normally hydrated female subjects after oral paracetamol (1·5 g), but the effect on urine osmolality was uncertain (Table 3). The changes in urinary excretion produced by acetylsalicylic acid in normally hydrated female subjects are compared to those which occurred following paracetamol in Fig. 3.

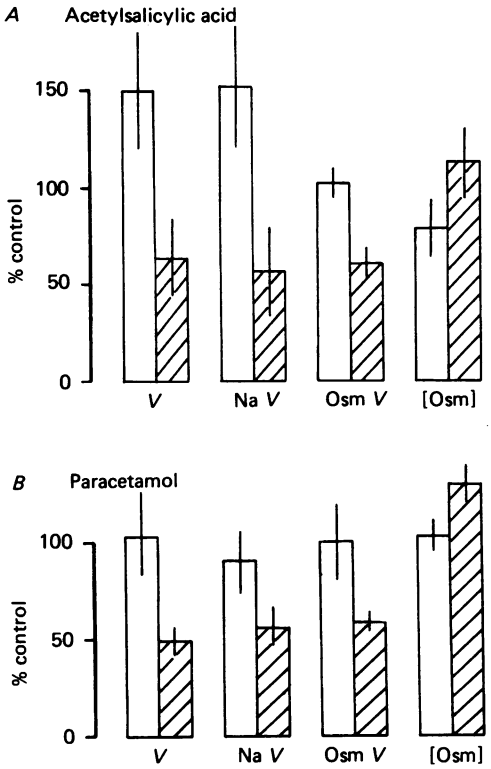

Fig. 3. Comparison of the effects of acetylsalicylic acid (*A*) and paracetamol (*B*) on urine flow (*V*), sodium output (Na *V*), osmolal output (Osm *V*) and urine osmolality (Osm) in normally hydrated female subjects. Each column represents measurements taken after treatment expressed as a percentage of the value obtained in the previous 3 hr. Hatched columns denote measurements taken following acetylsalicylic acid or paracetamol.

DISCUSSION

In the dog (Fulgraff & Brandenbusch, 1974) and the rabbit (Fine & Trizna, 1977) prostaglandin E_2, the major renal prostaglandin (Daniels, Hinman, Leach & Muirhead, 1967) exerts two prominent effects on renal function, i.e. vasodilatation and natriuresis. The major site of prostaglandin production in the kidney was originally thought to be the interstitial cells of the renal medulla from which prostaglandins can be synthesized in tissue culture (Muirhead, Germain, Leach, Pitcock, Stephenson, Brooks, Brosius, Daniels & Hinman, 1972), and the natriuretic properties of renal prostaglandins were suggested to be a consequence of their ability to increase renal

blood flow (McGiff & Itskovitz, 1973). The physiological control of prostaglandin synthetase in the kidney was therefore considered by correlating the intrarenal distribution and activity of prostaglandin synthetase with the potential roles for prostaglandin E_2 in the control of renal circulation, rather than in the control of urine composition (McGiff & Malik, 1976). In recent years, it has become evident that prostaglandin synthetase is not confined to medullary interstitial cells but is also located in the renal cortex (Larsson & Anggard, 1973; Smith & Bell, 1978) and in the medullary collecting duct (Janszen & Nugteren, 1971; Cavallo, 1976; Bohman, 1977; Smith & Wilkin, 1977). The importance of prostaglandin synthesis to the control of renal circulation may have to be reconsidered in the light of the absence of change in renal blood flow following the administration of prostaglandin synthetase inhibitors to conscious as opposed to anaesthetized animals (Swain, Heyndrickx, Boettcher & Vatner, 1975; Haylor & Lote, 1979).

In the present experiments, the renal excretion of sodium and water was reduced following the administration of prostaglandin synthetase inhibitors to healthy conscious humans. This response occurred in two groups of subjects under different conditions, i.e. normally hydrated females on an unrestricted diet and water-diuretic males receiving a saline infusion. The prostaglandin E content of urine was determined to assess whether renal prostaglandin synthesis had been effectively inhibited by the dose of acetylsalicylic acid used. The use of this indicator is based on evidence which suggests that the prostaglandin content of urine is derived entirely from the kidney (Frolich *et al.* 1975). Urinary PGE content was only measured in female subjects because in males it may be elevated by prostaglandin derived from accessory sex glands (Tan, Sweet & Mulrow, 1978). In normally hydrated female subjects, the presence of prostaglandin E in all urine samples collected over consecutive 3 hr periods suggested that the kidney in conscious humans continually synthesises prostaglandin, while the reduction in urinary PGE output which followed acetylsalicylic acid treatment indicated that renal prostaglandin synthesis was effectively reduced by the dose used (1·2 g orally). When an equivalent intravenous dose of acetylsalicylic acid (1 g) (Rowland & Reigelman, 1968) was given to water-diuretic male subjects the glomerular filtration rate (inulin clearance) and effective renal plasma flow (*p*-aminohippurate clearance) remained unaltered. A high urine flow must be established for accurate renal clearance studies to be performed, so such studies could not be carried out in normally hydrated female subjects. The lack of effect of aspirin on renal haemodynamics in water-diuretic subjects cannot necessarily be extrapolated to non-diuretic individuals. Conflicting results have been obtained for the effects of prostaglandin synthetase inhibitors on the glomerular filtration rate or effective renal plasma flow estimated from the clearance of endogenous substances, i.e. creatinine or using single-injection techniques (Beeley & Kendall, 1971; Burry & Dieppe, 1976; Donker, Arisz, Brentjens, van der Hem & Hollemans, 1976; Mountokalakis & Karambasis, 1977) in non-diuretic subjects. However it must be borne in mind that such estimates may be altered by other factors than changes in renal function including competition for renal secretion, red cell binding or protein binding.

It may be concluded from the present experiments in healthy conscious humans that the renal excretion of sodium and water is reduced following prostaglandin

synthetase inhibition, without measurable changes in the glomerular filtration rate or the effective renal plasma flow. It is tempting to suggest that the continual production of prostaglandin by the human kidney may help to maintain sodium and water excretion. Such a contention is supported by two additional observations. First, that in one subject whose urinary PGE output was not reduced following acetylsalicylic acid treatment, sodium excretion was little altered. Secondly, that paracetamol (1·5 g orally), which in the rabbit is equipotent to acetylsalicylic acid as an inhibitor of renal prostaglandin synthetase (Blackwell, Flower & Vane, 1975), also reduced sodium and water excretion in normally hydrated female subjects. At this dose paracetamol also reduces the urinary output of prostaglandin E from non-diuretic female subjects (Haylor, 1977).

The results of the present experiments concerning the antidiuretic and anti-natriuretic effects of acetylsalicylic acid are supported by Elliott & Murdaugh (1962) and Berg (1977). However these authors attributed such properties to other biochemical actions of acetylsalicylic acid besides prostaglandin synthetase inhibition; these included effects on adrenal steroids and the uncoupling of oxidative phosphorylation (Berg & Bergan, 1977). The suggestion that, in conscious man, renal prostaglandin synthesis may help to maintain solute and water excretion is supported by the antidiuretic properties of other non-steroidal anti-inflammatory agents, including indomethacin (Berl *et al.* 1977) and phenylbutazone (Meiers & Wetzels, 1964) which are also known to inhibit renal prostaglandin production (Blackwell *et al.* 1975). The natriuresis and diuresis associated with either the intravenous infusion of PGA (Carr, 1970) or disease states where urinary PGE output is elevated (Gill *et al.* 1976) are also consistent with this hypothesis.

An indication of the mechanism by which prostaglandin synthesis may help to maintain solute and water excretion can also be obtained from the present experiments. Following prostaglandin synthetase inhibition, renal solute and water excretion decreased without a measurable change in the glomerular filtration rate, suggesting that tubular re-absorption had increased. This change occurred while the effective renal plasma flow and therefore probably the total renal blood flow remained unaltered, implying that in conscious man renal prostaglandins can reduce tubular reabsorption by a non-haemodynamic mechanism. This response was unlikely to be part of the renal response to volume expansion as suggested by Dusing, Melder & Kramer (1976) as it could also be demonstrated in normally hydrated female subjects on an unrestricted diet. Volume expansion has been shown however to increase the renal venous outflow of prostaglandins in man (Papanicolaou *et al.* 1975). The tubular changes produced by prostaglandins have been attributed to their ability to antagonize the action of antidiuretic hormone (Grantham & Orloff, 1968), and in man indomethacin can potentiate the renal changes produced by ADH, i.e. increased urine osmolality and decreased free water clearance (Berl *et al.* 1977). In the present experiments using male subjects, acetylsalicylic acid did decrease free water clearance and increase urine osmolality, but these changes occurred during a water-diuresis where presumably very low levels of circulating ADH were present, and a change in urine osmolality was not observed following acetylsalicylic acid treatment in normally hydrated subjects. ADH-like changes in renal function may be equally well explained by an increase in the medullary concentration gradient

which has been demonstrated to occur following indomethacin treatment in the rat (Ganguli, Tobian, Azar & O'Donnell, 1977). It is suggested therefore that in conscious man, acetylsalicyclic acid may evoke changes in tubular reabsorption by inhibiting the synthesis of renal prostaglandins which exert a more direct action on the renal tubule, possible by altering its permeability to sodium, although an interaction with ADH cannot be excluded. Prostaglandins are involved in maintaining the permeability of isolated frog skin (Haylor & Lote, 1976, 1977) and permeability changes have been suggested to explain the tubular actions of acetylsalicylic acid in the anaesthetized dog (Ramsey & Elliott, 1967) and of indomethacin in the conscious rat (Haylor & Lote, 1979).

Finally histochemical studies, particularly in the rabbit and rat, using immunofluorescent antibodies (Smith & Wilkin, 1977) or by staining for peroxidase (Cavallo, 1976) indicate that the medullary collecting duct is the major part of the renal tubule which contains prostaglandin synthetase. In the medulla prostaglandins are also formed in the interstitial cells (Bohman, 1977; Zusman & Keiser, 1977) and two prostaglandin synthetases with differing pH optima have been isolated from medullary homogenates (Raz & Schwartzman, 1976). In the renal cortex prostaglandin synthesis is probably confined to arteriolar endothelial cells, although staining to cyclo-oxygenase antibodies has also been shown in the epithelial cells of Bowman's capsule in the rabbit and glomerular mesangial cells in the cow. The classical prostaglandins (E_2 and $F_{2\alpha}$) are not the only biologically active products of the cyclo-oxygenase system in the kidney; prostacyclin (PGI_2) is formed by all three cell types (Grenier & Smith, 1978; Whorton *et al.* 1978) and the renal synthesis of thromboxane has been demonstrated (Morrison, Nishikawa & Needleman, 1977). The finding that in man, as in the conscious rat, prostaglandin synthetase inhibitors reduce urinary excretion without damaging renal blood flow supports the view that the kidney may contain at least two functionally independent prostaglandin synthetases. This functional separation may be related to the anatomically distinct locations of prostaglandin synthetase. The demonstration that the human kidney can synthesize prostaglandin which does not contribute to renal haemodynamics does not preclude the possibility that prostaglandin synthesized at other sites in the kidney could influence renal vasculature. Rather, it suggests that the synthesis of such prostaglandin is not stimulated under the conditions of the present experiments. Circumstances have been described in man where urinary prostaglandin E output is high and prostaglandin synthetase inhibitors do reduce the glomerular filtration rate or renal blood flow or both, e.g. lupus erythematosis (Kimberley, Gill, Bowden, Keiser & Plotz, 1978). Under such conditions urinary excretion following prostaglandin synthetase inhibition could be influenced by both a direct tubular and a haemodynamic component.

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