Dissociation of renin-aldosterone and renal prostaglandin E during volume expansion induced by immersion in normal man

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Summary

1. The relationship of the renin-angiotensin-aldosterone axis with renal prostaglandin E is complex. Although studies have suggested that these two hormonal systems respond to experimental manipulations in a parallel manner, their interdependence has not been assessed fully during volume expansion. Since studies have demonstrated that in normal man the central hypervolaemia induced by water immersion to the neck produces a prompt and profound suppression of plasma renin activity and plasma aldosterone concentration without concomitant alteration of plasma composition, immersion afforded a unique opportunity to assess simultaneously the effects of central hypervolaemia on plasma renin activity, plasma aldosterone concentration and prostaglandin E excretion.

2. Seven normal subjects were studied twice while in balance on a diet containing 10 mmol of sodium/day, 100 mmol of potassium/day: with indomethacin administration (50 mg given every 6 h for five doses) and without indomethacin. Urinary prostaglandin E excretion was measured hourly and plasma renin activity and plasma aldosterone concentration at 30 min intervals.

3. Immersion was associated with a marked suppression of plasma renin activity (59 ± 7%) and plasma aldosterone concentration (55 ± 3%) with a return to pre-study values during the recovery hour. Concomitantly, urinary prostaglandin E excretion increased from 4.7 to a peak of 10.9 ng/min. Although administration of indomethacin lowered the basal rate of urinary prostaglandin E excretion and plasma renin activity, it did not prevent the subsequent augmentation of urinary prostaglandin E or the suppression of plasma renin activity and plasma aldosterone during the subsequent 4 h of immersion.

4. These results demonstrate a dissociation of renin-aldosterone and prostaglandin E during hypervolaemia and suggest that whereas prostaglandin E may constitute one of the major determinants of renin release clinically and experimentally, these two hormonal systems can be dissociated from each other in response to central volume expansion in man.

Key words: aldosterone, angiotensin, renal prostaglandins, water immersion.

Introduction

Several lines of evidence have suggested that the renin-angiotensin system and renal prostaglandins interact in a dynamic manner to maintain blood pressure and volume homeostasis. According to this hypothesis, activation of the renin-angiotensin system increases production of vaso-depressor prostaglandins, which may modulate the pressor action of angiotensin (McGiff, Crowshaw, Terragno & Lonigro, 1970; Aiken & Vane, 1973). Conversely, alterations in renal prostaglandin synthesis produce parallel changes in plasma renin activity (Frolich, Hollifield,
investigators have demonstrated that inhibition of endogenous prostaglandin synthesis results in a decrease in release of aldosterone (Tan & Mulrow, 1977; Zusman, Vinci, Bowden, Horwitz & Keiser, 1979), thus suggesting an important role for the renal prostaglandins in modulating the responsiveness of the renin–angiotensin–aldosterone axis.

The present studies were designed to explore further the inter-relationships between the renin–angiotensin–aldosterone and the renal prostaglandin systems in man. As previous studies in our laboratory have demonstrated that the central hypervolaemia induced by water immersion produce a prompt and profound suppression of the renin–aldosterone system without concomitant alterations of plasma composition (Epstein, Pins, Sancho & Haber, 1975), we undertook to assess the effect of an immersion-induced volume stimulus on the responsiveness of the renin–aldosterone system and simultaneously, on renal prostaglandin synthesis.

Methods

Seven normal male subjects between the age of 22 and 51 years were studied. None had a history of hypertension, cardiovascular disease or diabetes. Renal diseases were excluded in all subjects as they had normal urine sediments and creatinine clearances. During the study the subjects were in an environmentally controlled metabolic ward kept at a constant temperature. Throughout the study each subject remained on an unchanged diet, containing 10 mmol of sodium, 100 mmol of potassium and 2500 ml of water/24 h. Daily 24 h urine collections were made for determination of concentrations of sodium, potassium and creatinine. After dietary equilibration, each subject was studied during water immersion to the neck for 4 h (09.00-13.00 hours), preceded and followed by 1 h of quiet sitting outside the tank (pre-study and recovery hours respectively).

Each subject stood briefly to void spontaneously at hourly intervals during the study. To maintain adequate urine flow, 200 ml of water was given orally every hour during the study. Portions of each hourly urine specimen were frozen promptly for prostaglandin E determinations. Blood was collected at 30 min intervals for renin and aldosterone determinations. All subjects were weighed every morning at 07.00 hours, after voiding, and before and after each study.

Immersion was carried out in a water-proof tank as described by Epstein, Katsikas & Duncan (1973). A constant water temperature of 34.5 ± 0.5°C was maintained by two heat-exchangers, controlled by an adjustable temperature-calibrated control meter with the input derived from two thermistors immersed at different water levels. This temperature was previously found to produce optimal comfort for the subjects and to maintain a constant body core temperature (Epstein et al., 1973). Plasma renin activity was measured by the radioimmunoassay method of Haber, Koerner, Page, Kliman & Purnode (1969). Plasma aldosterone was measured by a radioimmunoassay technique which allowed the direct assay of aldosterone in plasma extracts without the need for prior fractionation (Sancho & Haber, 1978); a new antibody with a very high affinity constant (10^{-11} mol/l) was used, so that 50% displacement is obtained with only 8 pg of aldosterone. With this approach, aldosterone could be assayed in a 0.1 ml sample of plasma. The coefficient of variation for interassay determinations is 10-2%.
Urinary prostaglandin E was assayed by a radio-receptor assay with the extraction and assay procedures of Epstein, Lifschitz, Hoffman & Stein (1979). In brief, a 1 or 2 ml sample of urine was adjusted to pH 6-8 and extracted with benzene/butyl chloride (1:1, v/v). The pH was then adjusted to 3-5 with formic acid and the fatty acids (including prostaglandin E) were extracted with 20 ml of chloroform. The chloroform was then flash evaporated and the residue applied to a Sephadex LH 20 column. The appropriate fractions were collected and were assayed after being concentrated. The assay was carried out with rat liver membranes, which contain a class of receptors that specifically bind prostaglandin E, as confirmed by previous studies in this laboratory. Prostaglandin E₁ cannot be differentiated from prostaglandin E₂ as there is almost identical binding of these two compounds to these receptors; prostaglandins of the B and F series including 6-keto-prostaglandin F₁α demonstrate no measurable binding, and prostaglandin A₁ binds with approximately one-sixteenth the affinity of prostaglandin E₁. Recovery of unlabelled prostaglandin E₁ was determined over a concentration range of 200-10 000 pg and in nine separate samples, determined on different days, the measured values averaged 99 ± 9% of the predicted values. Samples from a given patient were all analysed at the same time. Although the major urinary prostaglandins are in the prostaglandin E₂ series, the present results are expressed as prostaglandin E because these receptors bind to prostaglandin E₁ and prostaglandin E₂ similarly. In the presentation of the results, mean values include the evaluated SEM as an index of dispersion. Results were evaluated statistically by paired or unpaired t-test analysis. Differences with P < 0.05 or greater were considered significant.

Permission for the study was obtained from each subject after a detailed description of the procedure and potential complications. The protocol was approved by the Human Experimentation Committees of the University of Miami School of Medicine and the Miami Veterans Administration Medical Center and complied with the principles set forth in the Declaration of Helsinki. There were no complications.

Results

The restriction of sodium intake to 10 mmol of sodium/day resulted in an exponential reduction in urinary sodium excretion. On the day before the initial immersion study, mean urinary sodium excretion was 9 ± 1 mmol.

Plasma renin activity

Fig. 1 depicts the changes in plasma renin activity during immersion and immersion + indomethacin. As can be seen, mean basal plasma renin activity was appropriately elevated in response to dietary sodium restriction during the pre-study period of immersion (5.6 ± 0.9 ng h⁻¹ ml⁻¹). During the study without indomethacin, immersion resulted in a significant suppression of plasma renin activity beginning within 30 min of study (P < 0.05 compared with pre-study). During the final hour of immersion, plasma renin activity attained nadir values of 2.1-2.2 ng h⁻¹ ml⁻¹, equivalent to 41 ± 7% of the pre-study value. Cessation of immersion was associated with a prompt return to pre-study values (P > 0.5 compared with pre-study).

Indomethacin administration resulted in a significant suppression of basal mean plasma renin activity during the pre-study period, before immersion, to 3.5 ± 0.7 ng h⁻¹ ml⁻¹ (P < 0.025 compared with the corresponding hour in the study without indomethacin). Despite the indomethacin-induced suppression of basal plasma renin activity, the subsequent 4-h period of immersion was still associated with a progressive suppression of plasma renin activity which mirrored the pattern of renin suppression observed during the immersion studies without indomethacin. Similarly, plasma renin activity increased promptly during the recovery hour of immersion + indomethacin from 1.3 ± 0.4 to 3.6 ± 0.8 ng h⁻¹ ml⁻¹ (P < 0.01).

Plasma aldosterone concentration

The changes in plasma aldosterone concentration observed during immersion and immersion + indomethacin are shown in Fig. 2. Mean basal plasma aldosterone was elevated in response to dietary sodium restriction during the pre-study period of the study without indomethacin (335 ± 63 pg/ml). Subsequently, immersion resulted in a significant suppression of plasma aldosterone beginning within 60 min of study (P < 0.025 compared with pre-study). Cessation of immersion was associated with a prompt return to pre-study values from 165 ± 27 to 345 ± 55 pg/ml (P < 0.01 compared with 240 min of immersion).

Indomethacin administration failed to alter significantly basal mean plasma aldosterone during the pre-study period of immersion +
Fig. 1. Effects of water immersion on plasma renin activity in seven normal subjects in balance on a diet containing 10 mmol of sodium/day and 100 mmol of potassium/day, with (O—O) or without ( ●—● ) indomethacin administration. Immersion resulted in a progressive suppression in plasma renin activity beginning as early as 30 min with attainment of nadir values during the final hour of immersion. Cessation of immersion was associated with a prompt return to pre-study values. Although indomethacin administration suppressed basal mean plasma renin activity significantly during the pre-study period ( \( P < 0.025 \) compared with immersion), the subsequent 4-h immersion period was still associated with a progressive suppression of plasma renin activity, which mirrored the pattern observed during immersion without indomethacin administration. *Significance of results on comparison with indomethacin: \( P < 0.05 \).

Urinary prostaglandin E excretion

Urinary prostaglandin E determinations were obtained hourly in the seven subjects during both the immersion and immersion + indomethacin studies. As shown in Fig. 3, immersion was associated with a progressive increase in urinary prostaglandin E excretion from 4.7 to peak values of 9.9 ± 2.2 and 10.9 ± 3.8 ng/min during hours 3 and 4 respectively. Cessation of immersion was associated with a prompt and marked (76%) decrement in urinary prostaglandin E excretion from 10.9 ± 3.8 to 2.6 ± 0.4 ng/min.

Indomethacin administration resulted in a 77% decrement in basal urinary prostaglandin E excretion during the pre-study hour preceding immersion + indomethacin ( \( P < 0.01 \) for immersion versus immersion + indomethacin). During the subsequent 4 h of immersion, urinary prostaglandin E values were three- to four-fold the pre-study values. Recovery was associated with a prompt decrement in urinary prostaglandin E from 3.9 ± 0.7 to 0.9 ± 0.2 ng/min ( \( P < 0.01 \) ). Although the patterns of urinary
FIG. 2. Effects of water immersion on plasma aldosterone concentration in seven normal subjects in balance on a diet containing 10 mmol of sodium/day and 100 mmol of potassium/day with (O—O) or without (□—□) indomethacin administration. Immersion resulted in a progressive suppression in plasma aldosterone concentration beginning as early as 60 min with attainment of nadir values during the final hour of immersion. Cessation of immersion was associated with a prompt return to pre-study values. Indomethacin administration did not alter the suppression of plasma aldosterone during the subsequent 4-h immersion period as compared with that observed during immersion without indomethacin administration. *Significance of results on comparison with indomethacin, $P < 0.05$.

Discussion

There is considerable evidence to suggest that there is a close association between the renin–angiotensin system and the renal prostaglandin system. Perhaps the earliest studies along these lines are those in which angiotensin II (ANG II) was infused into the renal artery of an isolated perfused kidney and prostaglandin E-like material was identified in the renal venous effluent by bioassay (McGiff et al., 1970). Studies in both the intact animal and man (Frolich, Wilson, Sweetman, Smigel, Nies, Carr, Watson & Oates, 1970) have shown that prostaglandin E excretion during immersion and immersion + indomethacin were similar, the urinary prostaglandin E excretion values during immersion exceeded those of immersion + indomethacin during 3 h of the 4 h period of immersion (Fig. 3).

Serum potassium concentration

In light of the dissociation between plasma renin activity and plasma aldosterone induced by indomethacin, serum potassium concentrations were compared during the immersion and immersion + indomethacin studies. The serum potassium concentration obtained immediately before the immersion study ($3.9 \pm 0.1$ mmol/l) was not different from the corresponding value immediately before the immersion + indomethacin study ($3.8 \pm 0.2$ mmol/l) ($P > 0.5$). Similarly, serum potassium concentration at the end of immersion ($4.0 \pm 0.1$ mmol/l) did not differ from a corresponding value during the immersion + indomethacin study ($4.1 \pm 0.2$ mmol/l) ($P > 0.5$).
Both of these drugs are potent inhibitors of cyclo-oxygenase (an early step in the synthesis of prostaglandins) but are quite different structurally. Indomethacin has been shown to be a potent inhibitor of renin release in man and animals (Larsson, Weber & Anggard, 1974; Frolich et al., 1976) and in the few studies where meclofenamate was used similar results were obtained (Romero et al., 1976).

Although fewer studies examining the relationship between renal prostaglandins and aldosterone secretion have been made, the available data suggest a similar parallelism between these two hormonal systems. Thus Zipser, Hoefs, Speckart, Zia & Horton (1979) have reported that the administration of inhibitors of prostaglandin synthetase (both indomethacin and ibuprofen) resulted in a lowering of plasma aldosterone concentration in addition to the effects on prostaglandin E$_2$ values and plasma renin activity expected in patients with decompensated cirrhosis. Tan & Mulrow (1977) have reported that administration of indomethacin blunted the stimulatory response of plasma and urinary aldosterone expected on frusemide challenge in a group of normal subjects.

Despite the wealth of data suggesting a parallelism between the renin–angiotensin–aldosterone system and renal prostaglandins, isolated observations from others (Berl, Henrich, Erickson & Schrier, 1979; Seymour & Zehr, 1979) and our own laboratory have questioned such a theory. Thus studies from this laboratory have demonstrated that water immersion to the neck results in a marked augmentation of prostaglandin E production in normal man (Epstein et al., 1979). Furthermore, earlier studies with a comparable immersion model demonstrated a suppression of plasma renin activity and aldosterone (Epstein et al., 1975; Epstein, Levinson, Sancho, Haber & Re, 1977). Although extrapolation of the results drawn from these two isolated observations would suggest that the two systems may be dissociated, such inferences must be drawn with great caution; the conditions of study, the experimental manipulations and the species used in earlier investigations differed markedly from those with normal man undergoing immersion. Furthermore, our earlier observations during immersion were not documented simultaneously in the same study population. The hypothesis of renin–aldosterone–prostaglandin parallelism should be tested simultaneously in identical subjects under carefully defined conditions of study. Thus, in the present study, we used the immersion model to characterize the simultaneous alterations in

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![Graph](image_url) **Fig. 3.** Effect of water immersion on renal prostaglandin E excretion in seven normal sodium-depleted subjects with (---) or without (••••) indomethacin administration. Immersion was associated with a progressive increase in urinary prostaglandin E excretion from 4.7 to peak values of 9.9 and 10.9 ng/min during hours 3 and 4 respectively. Recovery was associated with a prompt decrement to 2.6 ± 0.4 ng/min (P < 0.01). Although indomethacin administration was associated with a baseline urinary prostaglandin E excretion which was 77% lower than that observed during immersion, it did not prevent the subsequent augmentation of urinary prostaglandin E during immersion + indomethacin. *Significance of results on comparison with indomethacin: P < 0.05.
plasma renin activity, plasma aldosterone concentration and prostaglandin E excretion in sodium-depleted normal human beings. The present observations demonstrate conclusively that the experimental manipulation of water immersion dissociates the suppression of renin–aldosterone from the simultaneous augmentation of prostaglandin E excretion in normal subjects.

Interpretation of the present findings should consider whether urinary prostaglandin E excretion reflects renal prostaglandin E synthesis. Urinary prostaglandin E was measured because it is thought to be the best index of renal prostaglandin E production (Dunn & Hood, 1977). Studies in which both renal venous and urinary prostaglandin E were measured demonstrated parallel changes in both, but quantitatively more prostaglandin E was found excreted in the urine (Dunn et al., 1978). Although it has been suggested that urinary flow rate is a major determinant of urinary prostaglandin E excretion (Wright & Lifschitz, 1979), previous studies from our laboratory have shown that the increase in urinary prostaglandin E during immersion is not solely a function of increased urine flow rate, but rather reflects increased renal prostaglandin E production (Epstein et al., 1979).

Our results are not necessarily at variance with previous studies which support the hypothesis that the prostaglandin system is involved in at least some of the mechanisms of renin release. Rather, the present results suggest that whereas the prostaglandin system may be intimately involved in the mediation of renin release in a number of experimental systems, renal prostaglandins constitute merely one of several determinants of renin release.

Although several studies have suggested that the prostaglandin-dependent component of renin release is mediated by prostaglandin I₂ (Whorton, Misano, Hollifield, Frolich, Inagami & Oates, 1977; Gerber, Branch, Nies, Gerkens, Shand, Hollifield & Oates, 1978), Whorton, Lazar, Smigel & Oates (1980) have compared the efficacy of several prostaglandins and demonstrated an important role for prostaglandin E₂ in increasing renin secretion in renal cortical slices. Additional studies will be necessary to assess the relative contribution of prostaglandin I₂ and prostaglandin E₂ concentrations in mediating renin release in vivo. Clearly prostaglandin E₂ is capable of mediating renin release. Furthermore, most of the available data assessing the inter-relationship of the renin–angiotensin system and renal prostaglandins have been obtained by measuring prostaglandins of the E series, demonstrating that prostaglandin E₂ and the renin–angiotensin system respond in a parallel manner. The present study extends these observations, demonstrating that plasma renin activity and prostaglandin E excretion can be dissociated in normal man. Additional studies will be necessary to assess the relationship of plasma renin activity and prostaglandin I₂ excretion under similar conditions.

The results of the present study are important in the understanding of many earlier studies dealing with renin–prostaglandin interactions. Several such studies, which have examined the role of renal prostaglandins in a number of experimental systems, have involved the administration of prostaglandin synthetase inhibitors. Implicit in such an approach is the assumption that a resultant blockade would nullify the concentration of renal prostaglandins. The present study demonstrates that despite the indomethacin-induced suppression of basal urinary prostaglandin E excretion, the stimulus of immersion was still associated with a progressive increment in urinary prostaglandin E excretion. These results emphasize that the indomethacin-induced inhibition of prostaglandin synthesis is only partial and suggest that when presented with an adequate stimulus, such as water immersion, the kidney can still augment markedly its rate of prostaglandin E production despite the presence of some degree of cyclo-oxygenase inhibition.

The present demonstration of the dissociation between indomethacin-induced suppression of basal plasma renin activity and prostaglandin E excretion on the one hand, and the lack of suppression of basal plasma aldosterone concentration in response to indomethacin administration on the other hand, is of interest. Although previous studies have examined the effect of prostaglandin synthetase inhibition on aldosterone release, the experimental conditions were not comparable with those in the present study. Thus although Zipser et al. (1979) demonstrated a suppression of plasma aldosterone after indomethacin and ibuprofen administration, these observations were restricted to patients with decompensated cirrhosis with markedly elevated plasma aldosterone concentrations. The demonstration that serum potassium concentrations were comparable during the studies with and without indomethacin suggests that a change in potassium does not account for the failure of plasma aldosterone to be suppressed after indomethacin administration. Additional studies are necessary to define the inter-relationship between renal prostaglandins and aldosterone in man.

In summary, the present studies demonstrate
that immersion-induced central volume expansion is associated with a marked increase in renal prostaglandin E excretion and a concomitant suppression of plasma renin activity and plasma aldosterone concentration. Whereas administration of indomethacin lowers both the basal rate of prostaglandin E excretion and basal plasma renin activity, it does not prevent the opposite responses found during immersion between prostaglandin E on the one hand, and plasma renin activity and plasma aldosterone concentration on the other. These observations are consistent with the hypothesis that, whereas prostaglandin E may be one of the stimuli for renin release, these two hormonal systems are not associated with each other in the response to central volume expansion in man.

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References


