The effect of mineralocorticoid administration on urine free dopamine in man

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(Received 1 June 1979; accepted 17 September 1979)

Summary

1. Five normal subjects were studied under metabolic conditions on a controlled sodium and potassium intake.
2. Plasma and urine free dopamine concentrations were measured in these subjects before, during and after 5 days administration of fludrocortisone (0.2 mg twice daily).
3. Urine free dopamine showed a tendency to fall during the early phase of fludrocortisone administration and then rose towards normal.
4. In a patient with primary hyperaldosteronism there was no evidence of increased renal production of dopamine. Urine dopamine fell when plasma renin activity rose as a result of spironolactone administration (200 mg three times a day for 5 days).
5. If renal dopamine has a role in mineralocorticoid ‘escape’ then it may be permissive only. The mechanisms of control of dopamine production could include tubular sodium concentration, tubular chloride concentration and intrarenal renin activity.

Key words: dopamine, kidney, mineralocorticoid, sodium.

Introduction

Our previous studies have demonstrated that the administration of sodium chloride to rats and man increases urine dopamine but leaves plasma dopamine unchanged (Ball, Oates & Lee, 1978; Oates, Ball, Perkins & Lee, 1979). This has led to the concept that dopamine could be an intrarenal natriuretic (or chloriuretic) hormone (Cuche, Kuchel, Barbeau, Boucher & Genest, 1972; Oates et al., 1979).

When normal man is given a powerful mineralocorticoid, there is an initial, rather variable, period of sodium retention followed by a period when sodium excretion rises towards dietary intake, despite continued mineralocorticoid administration. This return to normal sodium excretion has come to be known as mineralocorticoid ‘escape’ and much research effort has been made to identify the factor or factors responsible for the sodium loss. Factors which have been suggested include physical forces within the kidney, such as the peritubular oncotic pressure (Schrier & de Wardener, 1971), and/or the production of natriuretic substances such as kallikrein (Margolius, Horwitz, Geller, Alexander, Gill, Pisano & Keiser, 1974), small peptides (Schrier & de Wardener, 1971) and the prostaglandins (Nasjletti, McGiff & Colina-Chourio, 1978).

Kuchel, Buu, Unger & Genest (1978) have suggested that in primary hyperaldosteronism the urinary excretion of dopamine is increased and that this catecholamine may have a functional role in the production of renal escape from mineralocorticoid. In an attempt to confirm these observations we administered fludrocortisone to normal subjects under defined metabolic conditions and measured changes in plasma and urine dopamine concentrations.
Methods

Experimental subjects

Study A. Five male volunteer students who had given informed consent were admitted to a metabolic unit for 2 weeks. All meals were taken in the unit and alcohol was forbidden. Throughout the experiment they received a diet containing 120 mmol of sodium and 50 mmol of potassium each day. After 3 days adjustment to this diet they were given fludrocortisone, 0.2 mg twice daily, at 08.00 and 20.00 hours, for 5 days. At the end of 5 days, fludrocortisone was stopped and the metabolic balance continued for a further 3 days. The protocol had previously been approved by the Ethics Committee of the Leeds General Infirmary.

Study B. A sixth volunteer student (N.J.) was found, on physical examination, to have minimal aortic stenosis and it was therefore felt unwise to administer mineralocorticoid in view of the risk of cardiac failure. He remained in the metabolic unit for the whole period of the experiment and continued the daily intake of 120 mmol of sodium and 50 mmol of potassium throughout the 11 days.

Study C. A 38-year-old male with sustained hypertension and hypokalaemia (mean blood pressure 180/125 mmHg; mean plasma potassium 3.2 mmol/l) had the diagnosis of primary hyperaldosteronism confirmed by raised urine and plasma aldosterone concentrations together with a raised aldosterone concentration in the left adrenal vein. An adenoma was subsequently removed from the left adrenal gland. Before operation the patient was admitted to a metabolic unit to study the effect of spironolactone on body weight, plasma renin activity, blood pressure and 24 h urinary excretion of sodium, potassium and dopamine. Initially the patient was started on a diet based upon his normal electrolyte intake containing 150 mmol of sodium and 50 mmol of potassium/day and, after stabilization, spironolactone was administered, 200 mg three times a day, for a period of 5 days. The diet was continued as before. Spironolactone was then stopped and the study proceeded for a further 7 days. Blood pressure during the course of spironolactone administration fell from 180/120 mmHg to 140/95 mmHg and then rose to pretreatment levels after spironolactone had been discontinued.

Analytical methods

Blood samples for the determination of dopamine, plasma renin activity, creatinine, sodium, potassium, urea and electrolytes, haemoglobin and packed cell volume were taken each morning at 08.00 hours before rising. For dopamine estimation 20 ml of blood was collected into 400 μl of ethanediobis(ethyamine)tetra-acetic acid (EGTA, 0.2 mol/l) and ascorbic acid (0.06 mol/l) contained in tubes in ice. For the measurement of plasma renin activity, portions (5 ml) of blood were added to tubes in ice, each containing 100 μl of the disodium salt of ethylenediamine tetra-acetic acid (EDTA, 0.13 mol/l). Blood (10 ml) was taken in the routine way for the other investigations. The blood samples for dopamine and plasma renin activity were centrifuged at 2000 g for 15 min at 4°C and the plasma was removed.

Dopamine was extracted immediately at pH 8.3 from 10 ml of plasma by using 200 mg of alumina previously treated with 5 ml of EDTA (0.2 mol/l) and the dopamine eluted with 1 ml of acetic acid (0.2 mol/l) (Nagatsu, 1973; Ball et al., 1978). The dopamine was then measured by a radioenzymatic procedure with S-[3H]adenosyl-methionine and rat liver catechol-o-methyltransferase. The methylated amines were separated by thin-layer chromatography on silica gel and the radioactive 3-methoxytyramine was measured by liquid scintillation counting (Da Prada & Zürcher, 1976). Plasma renin activity was measured by the method of Haber, Koerner, Page, Kliman & Purnode (1969), with a modified incubation procedure at pH 5.7 and an additional enzyme inhibitor, phenyl-methylsulphonyl fluoride.

Urine (24 h specimen) was collected each day from 08.00 to 08.00 hours into bottles containing sufficient hydrochloric acid (3 mol/l) to ensure that the pH of the collected urine did not rise above 3, in order to avoid oxidation of dopamine in alkaline conditions. A portion of urine was taken for the measurement of sodium, potassium and creatinine by standard techniques and a further 5 ml sample was used for dopamine estimation.

The catecholamines were immediately extracted by using alumina (5 ml of urine to 500 mg of alumina), as described above, and eluted into acetic acid (3 ml) for dopamine measurement by the radioenzymatic technique. With this modification of the method of Da Prada & Zürcher (1976) the recoveries of added dopamine from plasma or urine were almost identical (64 ± 4.7%, plasma; 63 ± 5.8%, urine, Oates et al., 1979) and the values were compared directly. Corrections have not been made to the values given in the Results section for absolute recovery. The lower limit of sensitivity of this method was 0.13 nmol/l of plasma (or of urine).
All the statistical comparisons were made by the t-test for paired data.

Results

Study A

The general metabolic results of study A are shown in Fig. 1. When fludrocortisone was administered there was a striking fall in urine sodium ($P < 0.01$, day 1 vs day 3) and a less convincing rise in urine potassium ($P > 0.1$, day 1 vs day 3). Body weight also rose and reached a maximum on the third day of fludrocortisone administration, but this change was not significant ($P > 0.2$). The fall in urine sodium was not sustained and by day 5 of fludrocortisone administration sodium output was significantly greater than day 1 of mineralocorticoid administration ($P < 0.02$) but not significantly different from control day 1 ($P > 0.1$). Body weight fell on continued fludrocortisone administration although this change was not significant ($P > 0.1$, day 3 vs day 7).

On stopping fludrocortisone body weight continued to fall, but not significantly ($P > 0.1$, day 5 vs day 9). However, there was a sharp rebound increase in sodium excretion ($P < 0.001$, day 3 vs day 9; $P < 0.02$, day 7 vs day 9). A similar sharp fall in potassium excretion was also demonstrated ($P < 0.001$, day 3 vs day 9; $P < 0.001$, day 7 vs day 9).

Plasma renin activity is shown in Fig. 2. On administration of fludrocortisone, plasma renin activity fell progressively and by 72 h had fallen to a mean value of 0.27 ng h$^{-1}$ ml$^{-1}$, which was significantly lower than on days 1 and 2 ($P < 0.01$, day 1 vs day 5; $P < 0.01$, day 2 vs day 5). Plasma renin activity then remained low throughout the rest of the study. Mean systolic blood pressure on day 1 of the study was $121 \pm 3.6$ mmHg, diastolic pressure was $71 \pm 4.0$ mmHg. On day 6, the last day of fludrocortisone administration, mean systolic blood pressure was $122 \pm 3.4$ mmHg and mean diastolic pressure $72 \pm 3.9$ mmHg. There was no significant difference between the blood pressure during the control period compared with blood pressure during fludrocortisone administration.

Plasma dopamine concentrations are shown in Fig. 3. Plasma dopamine changed very little throughout the whole experiment and none of the changes observed was significant. The mean plasma dopamine on day 1 was 0.44 nmol/l; on day 3, 0.44 nmol/l; on day 7, 0.45 nmol/l; on day

\[ 8, \ 0.44 \text{ nmol/l} \quad (P > 0.1 \text{ for all comparisons}). \]

The results for plasma dopamine on the control days compare well with those reported previously by our group (mean 0.46 nmol/l, Oates et al., 1979).

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**Fig. 1.** Mean changes (±SEM) in body weight and excretion of sodium and potassium in five normal subjects before, during and after the oral administration of fludrocortisone (0-2 mg twice daily for 5 days). The broken lines indicate the daily oral intake of sodium and potassium (120 and 50 mmol respectively).

**Fig. 2.** Mean plasma renin activity (±SEM) in five normal subjects before, during and after the oral administration of fludrocortisone (0-2 mg twice daily for 5 days). Plasma samples were taken at 08.00 hours before rising. The subjects received the standard sodium and potassium diet throughout (120 and 50 mmol/day respectively).
Urinary dopamine excretion is also shown in Fig. 3, both for individuals and for the group as a whole. Urine dopamine tended to fall during the first 2 days of fludrocortisone administration and then to rise slowly towards or slightly above control values. The timing of the initial fall varied from subject to subject and although the pattern of the response seems clear from the mean values the changes were not significant (P > 0.05, day 1 vs day 3, day 1 vs day 7 and day 3 vs day 7). On stopping the fludrocortisone urine dopamine rose slightly, particularly in two subjects, and this change was significant at the 0.05 level (day 3 vs day 8).

Study B

Subject N.J.'s longitudinal results over the 10 day period are shown in Fig. 4. His mean plasma dopamine was 0.51 nmol/l (extreme range 0.48–0.54 nmol/l) and his mean urine dopamine was 1.09 μmol/24 h (extreme range 1.03–1.15 μmol/24 h). Mean plasma renin activity was 1.46 ng h⁻¹ ml⁻¹ (extreme range 1.06–1.80 ng h⁻¹ ml⁻¹).

Study C

The results on the patient with primary hyperaldosteronism are shown in Fig. 5. Urinary dopamine output, initially within our normal range for subjects on a controlled sodium diet, did not alter significantly during the period of spironolactone administration but fell steadily, as plasma renin activity reached a peak, 3 days after spironolactone had been discontinued. During the rebound from spironolactone, urinary dopamine appeared to parallel a falling urinary sodium output. However, this relationship was not seen in the last few days of spironolactone administration when increased urinary sodium excretion was not accompanied by an elevated urinary dopamine output. The responses of body weight, urine sodium and potassium and of plasma renin activity were similar to those reported previously (Spark & Melby, 1968) when the action of aldosterone is opposed at the renal receptor by spironolactone.

Discussion

Our recent studies suggest that urinary dopamine is formed largely in the kidney and that dopamine output in the urine increases markedly when
Fludrocortisone and urine dopamine

Fludrocortisone and urine doparnine

Fludrocortisone and urine doparnine

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Fludrocortisone and urine doparnine

Spironolactone

Kuchel et al. (1978) have suggested that urinary free and conjugated dopamine are increased in patients with proven primary hyperaldosteronism. The results reported here contradict those of Kuchel and his co-workers in two important respects. In the first place, administration of fludrocortisone failed to increase urine free dopamine and if anything free dopamine excretion fell initially. Secondly, in the subject with primary hyperaldosteronism, there was no evidence of increased urine free dopamine in the period before he received spironolactone.

We have included the results of subject N.J. to show the longitudinal reproducibility of the method for the measurement of plasma and urine dopamine. Of interest, urine dopamine for the group crosses N.J.'s results on day 5 of the study (day 3 of fludrocortisone), suggesting that normal urine dopamine is restored by this day. After this period of mineralocorticoid administration, normal subjects have usually entered the 'escape' period, although the mechanism by which this occurs is not fully understood. From our results urine dopamine would appear to have returned to normal values by this time and could, therefore, contribute to the increased sodium excretion.

Another factor that has been suggested to have a role in the renal adjustment to mineralocorticoid administration is increased production of urinary kallikrein. However, kallikrein output rises immediately on administration of fludrocortisone (Margolius et al., 1974) several days before increased sodium excretion. Urinary kallikrein activity is also raised in untreated primary hyperaldosteronism (Lechi, Coci, Lechi, Corgnati, Arosio, Zatti & Scuro, 1978) but whether this increase assists sodium excretion is disputed.

It has also been postulated that the renal prostaglandins have a role in the acquired resistance to mineralocorticoid, but recent work, in which prostaglandin synthesis was largely prevented by indomethacin, does not support this proposition (Zisper, Zia, Stone & Horton, 1978). However, with the same animal model, aprotinin, a known inhibitor of the kallikrein system, reduced sodium excretion when given 10 days after continuous deoxycorticosterone administration (Nasjletti et al., 1978). Large- and small-molecular weight peptides may be involved in the response of the kidney to salt and volume, but at present quantitative analysis of their function in this respect remains difficult (Clarkson, Raw & de Wardener, 1976).

Our experiments would suggest that initially dopamine tends to fall and this could limit the ability of the renal blood vessels to vasodilate and also the ability of the renal tubules to excrete sodium. By day 4 or day 5 of fludrocortisone administration several factors could summate to facilitate the excretion of sodium. Kallikrein will have risen to 200–300% of normal values; renin will have fallen to less than 10% of normal values and urine dopamine will have returned to normal concentrations. It may be that all three of these factors are required for maximum efficiency of the escape mechanism and might suggest that renal dopamine has a permissive action in this phenomenon. Further studies are needed to compare the efficiency of escape in animals (or subjects) receiving inhibitors of dopamine formation such as

![Fig. 5. Body weight, urine excretion of sodium, potassium and dopamine and plasma renin activity in a subject with primary hyperaldosteronism before, during and after the administration of spironolactone (200 mg three times a day for 5 days). Plasma samples were obtained at 08.00 hours before rising. The broken lines indicate the daily oral intake of sodium and potassium (150 and 50 mmol respectively).](image-url)
carbidopa (Ball & Lee, 1977) or specific dopaminergic antagonists, such as haloperidol (Yeh, McNay & Goldberg, 1969).

There may well be two main controlling influences on urinary dopamine output: the first could be the concentration of sodium (and/or chloride) ion in the renal tubule (Ball et al., 1978; Oates et al., 1979), and the second a rising intrarenal renin concentration, which, through angiotensin, could inhibit renal production of dopamine. The kidney may be able to distinguish between stimuli associated with volume expansion, resulting in increased local kallikrein production, and stimuli associated with an elevated sodium or chloride concentration in the renal tubule, producing increased local dopamine formation.

In any event we have found no definite evidence for significantly increased dopamine excretion during mineralocorticoid administration in subjects entering the escape period. If dopamine has a role in this phenomenon it may be permissive, in relation to other postulated factors such as kallikrein and the natriuretic peptides.

Acknowledgments

N.S.O. is supported by the Medical Research Council; C.M.P. by Sandoz Pharmaceuticals. We also thank the students who took part in the experiments, the nursing staff and dietitians of the Metabolic Unit at the Leeds General Infirmary and the Department of Chemical Pathology of the University of Leeds.

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