Plasma and urine dopamine in man given sodium chloride in the diet

N. S. OATES, S. G. BALL*, C. M. PERKINS AND M. R. LEE
Departments of Medicine and Pharmacology, The University of Leeds, Leeds, U.K.

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Summary

1. Plasma and urine free dopamine were measured daily for 5 days in six normal subjects maintained on a low sodium diet. The subjects were then given dietary supplements of sodium chloride for 5 days and the measurements repeated.

2. Throughout the experiment the 24 h free dopamine excretion rates for all the subjects were higher than could be accounted for by renal clearance. Dopamine excretion increased significantly in response to the added sodium chloride whereas plasma dopamine remained unchanged. The rise in dopamine excretion preceded that of sodium excretion.

3. It is concluded that free dopamine is formed within the kidney in response to increased dietary sodium and may have a role in the control of sodium excretion.

Key words: dopamine, kidney, sodium.

Abbreviation: PRA, plasma renin activity.

Introduction

Dopamine is a precursor of the other catecholamines found in man but in addition may have important actions of its own (Thorner, 1975), including a role in the control of sodium excretion by the kidney (Cuche, Kuchel, Barbeau, Boucher & Genest, 1972; Alexander, Gill, Yamabe, Lovenberg & Keiser, 1974; Ball & Lee, 1977a,b; Ball, Oates & Lee, 1978b).

Correspondence: Dr M. R. Lee, Department of Medicine, The General Infirmary, Great George Street, Leeds, LS1 3EX, U.K.

* Present address: MRC Blood Pressure Unit, Western Infirmary, Glasgow, Scotland, U.K.

Free dopamine is found in urine in amounts greater than can be accounted for by the total clearance of dopamine from the plasma perfusing the kidneys, suggesting that dopamine is formed in the kidney (Ball et al. 1978b). The rate of excretion and kidney tissue concentrations of dopamine also rise in response to increased dietary sodium, indicating increased formation of dopamine in the kidney (Alexander et al. 1974; Ball et al., 1977b; Ball, Lee & Oates, 1978a). We have studied the relationship between plasma and urine free dopamine under different conditions of salt load in man.

Method

Six male medical student volunteer subjects 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. They were given a diet containing 100 mmol of sodium and 50 mmol of potassium/24 h for 3 days, whilst establishing their daily routine, and then changed to a diet containing 20 mmol of sodium and 50 mmol of potassium/24 h (Fig. 1). After 5 days they continued on the same diet but with 200 mmol of extra sodium given as Slow Sodium (Ciba) in four equal doses, for a further 5 days (Fig. 1). The protocol had been previously approved by the Ethics Committee of the Leeds General Infirmary.

Blood samples for determinations of dopamine, plasma renin activity (PRA), creatinine, sodium, potassium, urea and electrolytes, haemoglobin and packed cell volume were taken each morning at 08.00 hours, before rising. For the dopamine estimation 20 ml of blood was collected into 400 ml of ethanedioxybis(ethylamine)tetra-acetic acid
(EGTA, 0.2 mol/l) and ascorbic acid (0.06 mol/l) contained in tubes in ice. For the measurement of PRA 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 PRA 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 1 ml of EDTA (0.06 mol/l) and the dopamine eluted with 1 ml of acetic acid (0.2 mol/l) (Nagatsu, 1973; Ball et al., 1978b). The dopamine was then measured by a radioenzymatic procedure using S-[3H]adenosylmethionine 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 & Züircher, 1976). PRA was measured by the method of Haber, Koerner, Page, Kliman & Purnode (1969), a modified incubation procedure at pH 5.7 with an additional enzyme inhibitor, phenylmethylsulphonyl fluoride, being used.

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, 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. A further 10 ml sample was used for dopamine estimation.

The catecholamines were immediately extracted from the urine by using alumina (10 ml of urine to 500 mg of alumina) as described for plasma and eluted into acetic acid (0.2 mol/l; 3 ml) for dopamine measurement by the radioenzymatic technique. With these methods the recovery of added dopamine from plasma or urine is the same (64 ± 4.7%, plasma; 63 ± 5.8%, urine) and the values can be compared directly. No correction is made for absolute recovery in the values given in the Results section. The lower limit of sensitivity of this method is 0.13 nmol/l of plasma (or urine).

All the statistical comparisons were made by paired t-test.

**Results**

During the period of low salt diet all the subjects rapidly came into sodium balance by reducing sodium excretion to equal their dietary intake (Fig. 1). On addition of sodium chloride to the diet, urinary sodium excretion rose only slightly on the first day but then increased markedly to reach a maximum by the fourth day. Sodium excretion was significantly higher on days 2, 3, 4 and 5 ($P < 0.01$) of the high sodium period compared with any day in the low salt period.

Dopamine excretion showed little change during the low salt period and increased only slightly on the first day of increased dietary sodium (Fig. 1). By the second day of increased sodium intake there was a marked increase in dopamine excretion, which was at a maximum on this day and decreased on subsequent days. Dopamine excretion was significantly higher on days 2 and 3 ($P < 0.01$) and on days 4 and 5 ($P < 0.05$) of the increased sodium period compared with any day on the low salt diet.

![Graph showing sodium excretion, PRA, dopamine excretion and creatinine clearance](image_url)

**Fig. 1.** Mean 24 h sodium excretion, plasma renin activity (PRA), 24 h dopamine excretion, plasma dopamine, dopamine clearance and creatinine clearance for the six subjects for each day during the low sodium period (20 mmol/day for 5 days) and the high sodium period (low sodium diet with 200 mmol of sodium added each day as Slow Sodium (Ciba)). Plasma samples were taken at 08.00 hours each morning and the mean results (± SEM) are plotted at the beginning and the end of each 24 h period.
Dopamine clearance showed a similar pattern to dopamine excretion (Fig. 1). Dopamine clearance reached a maximum on the second day of the high salt diet and was significantly higher on this day ($P < 0.01$) and on day 3 ($P < 0.02$) of high salt diet than on any day in the low salt period. Although dopamine clearance remained higher on days 4 and 5 of the high salt diet, compared with any day in the low salt period, the differences were not significant.

Plasma dopamine concentrations were remarkably stable both in the same individual on different days and for the group as a whole throughout the experiment (Fig. 1). The lowest plasma dopamine in any individual in the group was 0.37 nmol/l and the highest in any individual was 0.57 nmol/l. The widest range of plasma values shown by any one subject was 0.41-0.57 nmol/l. No individual showed a systematic increase in plasma dopamine on the high sodium intake.

PRA increased in response to the low salt diet, reaching a maximum on the morning of day 3 of low sodium (Fig. 1). On addition of sodium to the diet PRA decreased. PRA was significantly lower on days 2, 3, 4 and 5 of high salt compared with any day on the low salt diet ($P < 0.05$). Creatinine clearance showed no sustained changes over the course of the experiment (Fig. 1).

**Discussion**

The introduction of radioenzymatic techniques has enabled accurate measurement of the plasma catecholamines (Callingham & Barrand, 1976), most techniques being developments of the method of Engelman & Portnoy (1970), which is based on the production of the 3-O-methylated derivatives from the amines after reaction with the methyl-3H- or methyl-14C-labelled donor S-adenosylmethionine and a partially purified preparation of catechol-O-methyltransferase. Thin-layer chromatography is then used to separate the methylated products (Engelman & Portnoy, 1970; Christensen, 1973; Da Prada & Zürcher, 1976).

We used the method of Da Prada & Zürcher (1976), with an additional alumina extraction step. No important inhibition of catechol-O-methyltransferase by alumina could have occurred, as when corrected for recovery, our reported values are similar to those of Da Prada’s group (Da Prada & Zürcher, 1976; Bühler, Da Prada, Haefely & Picotti, 1978; Ball et al. 1978b). The method is highly specific for the catecholamines, although it has been reported that the measurement of dopamine can be interfered with by dopa which has been converted into dopamine by dopa decarboxylase contaminating the crude catechol-O-methyltransferase preparation (Ben-Jonathan & Porter, 1976). However, Da Prada & Zürcher (1976) found that dopa did not interfere with the measurement of dopamine under the conditions of their assay.

We made serial measurements in the same subjects over a period of 10 days. This not only allowed direct comparison of the plasma and urine values, but also an examination of their relationship in response to increased dietary sodium.

Our findings that urinary free dopamine greatly exceeds plasma free dopamine, and calculated clearance values far exceeded renal plasma flow, confirmed our previous studies in normal man (Ball et al. 1978b), all indicating that free dopamine is formed within the kidney. The increase in urinary dopamine excretion, in the absence of changes in plasma dopamine after increased dietary sodium, implies that free dopamine is formed within the kidney in response to sodium. The excretion of dopamine reached its maximum on the second day of salt loading and showed a fall on the subsequent days, whereas sodium excretion did not reach its maximum value until the fourth day. This suggests that dopamine leads, rather than follows, sodium excretion. The subsequent falls in both dopamine and sodium excretion may indicate that a new equilibrium state is being established with raised dopamine and sodium excretion rates.

In contrast to the response to a high salt intake, dopamine excretion showed little change in the low salt period (Fig. 1). Further evidence for increased renal dopamine formation in response to a high salt intake is that kidney concentrations of dopamine rose in rats given a high sodium intake (Ball et al., 1978a).

PRA changed as expected, increasing in the low salt period to a maximum on the morning of the third day, and then subsequently falling as the subjects adapted to their continued low salt intake. The increased dietary sodium rapidly suppressed PRA, mirroring the changes in dopamine excretion.

Free dopamine seems to be formed within the kidney and its production rises in response to an increase in dietary sodium. Numerous substances, including kallikrein (Adetuyibi & Mills, 1972) and the prostaglandins (Attallah & Lee, 1973), have been proposed as intrarenal natriuretic agents. How dopamine relates to these, if at all, is not known. As renin may have a role within the kidney to retain sodium (Lee, 1969), so dopamine may
form an integral part of the sodium excretory mechanism.

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References


BALL, S.G. & LEE, M.R. (1977a) Increased urinary dopamine in salt loaded rats. Clinical Science and Molecular Medicine, 52, 20P–21P.


THORNER, M.O. (1975) Dopamine is an important neurotransmitter in the autonomic nervous system. Lancet, i, 662–665.