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Factors affecting the release and excretion of dopamine in the rat

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The effects of inorganic salts and of diuretic agents on the excretion of dopamine (DA) were examined in the rat. Both types of treatment evoked significant increases in urinary DA excretion, urine volume and urinary sodium excretion. In the salt-treated animals a significant correlation was observed between DA excretion and urine volume, whereas after diuretic treatment there was better correlation between DA and urinary sodium excretion. The salt-treated animals showed a high correlation between administered chloride and DA excretion. Subcutaneous administration of DA produced a significant diuresis and an increase in sodium excretion. The mechanisms responsible for these responses are discussed with reference to the possibility that DA has a physiological role in the kidney.

It is well established that in the brain (Hornykiewicz 1966) and the cardiovascular system (Horwitz et al 1962) dopamine (DA) can have actions independent of its precursor role in the synthesis of noradrenaline (NA). Evidence has rapidly accumulated that DA also has an independent function in the kidney (Meyer et al 1967; Goldberg 1972).

DA, which occurs in concentrations of 0·02 to 0·04 $\mu g\ g^{-1}$ in the kidney of rats, guinea-pigs, dogs and cats (Anton & Sayre 1964), is the only endogenous catecholamine known to dilate renal vasculature (Halushka & Hoffman 1968). Metabolic studies show an excretion of free DA 10 to 20 times that of free adrenaline and NA; while the excretion of DA metabolites is about equal to that of the sum of the metabolites of adrenaline and NA (Thorner 1975).

The fact that excretion of free DA in the urine cannot be accounted for merely by the clearance of DA from the circulation has led to the suggestion that DA is synthesized in the kidney (Alexander et al 1974; Ball et al 1978). This view is supported by the fact that DA-containing neurons have been identified in the renal cortex of the dog (Bell et al 1978; Dinerstein et al 1978). Attention has been centred on the direct and significant correlation existing between the concentration of urinary sodium and DA in man (Alexander et al 1974; Ball et al 1978) however, this relationship is not specific to the sodium ion (Ball et al 1978).

We have examined the effects of inorganic salts and of diuretic agents on the urinary excretion of DA in the rat, a species in which the role of renal DA has not been widely investigated.

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MATERIAL AND METHODS

Male Wistar rats (University of Bath Strain, 300–350 g), individually housed in metabolism cages allowing separation of urine and faeces, were given free access to Oxoid 41B pellet diet and water. Acclimatization to the cages over 3 days was denoted by a constant intake of food and water and a regular weight gain. In the same time animals also became accustomed to handling and to the use of an oral needle.

Inorganic salt administration

Seven groups of 10 rats were used. Two groups, one of which was water-loaded (20 ml kg^{-1}) served as controls. Other groups were given 20 ml kg^{-1} of NaCl, KCl, NH_4Cl, $NaHCO_3$ or $CaCl_2$ by mouth at 1% for 3 days and 3% for a further 3 days. The effects of salt intake on urine pH were observed on days one and four. 24 h urine samples were collected in sufficient 2 M $HClO_4$ to reduce the pH to < 3. Urinary DA, Na^+ and K^+ were assayed on days 2, 3, 5 and 6. In the calculation of the results, the volume of added $HClO_4$ was subtracted from the total 24 h urine volume.

Diuretic administration

Groups of 10 rats were used. The control group was water-loaded (20 ml kg^{-1} orally), to the others, frusemide, hydrochlorothiazide and triamterene were administered in doses of 30 and 100 mg kg^{-1} in 20 ml kg^{-1} water orally, and 6 h urine samples were collected in the presence of $HClO_4$ and assayed for DA, Na^+ and K^+.

Assay of DA (modification of Anton & Sayre 1964). Concentrated 11·6 M $HClO_4$ was added to a 5 ml

sample of urine in a 10 ml polyethylene centrifuge tube to give a final concentration of 0·4 M. The tube was capped and shaken vigorously for 5 min in a mechanical shaker. After centrifugation of the sample at 30000 *g* for 20 min at 10 °C in an MSE65 Ultracentrifuge, the clear supernatant was removed, adjusted to 10 ml with 0·4 M $HClO_4$ and poured into a beaker containing 400 mg activated alumina and 200 mg EDTA then stirred for 1 min and brought to and maintained at pH 8·3 with 5 M NaOH. Stirring was continued for a further 3 min and after separation by centrifugation, the precipitated alumina was washed 4 times with deionized water. After which the DA was eluted by vigorous shaking of the alumina for 15 min with 3 ml 0·05 M $HClO_4$. The supernatant, containing the DA was transferred to a small polyethylene centrifuge tube and spun at 30000 *g* at 10 °C for 20 min.

To 0·6 ml of the eluate was added 0·03 ml ethanol, 0·3 ml phosphate buffer (0·5 M made by adjusting a 1 M solution of K H_2PO_4 to pH 7·0 with 1 M NaOH and then diluting with deionized water to 0·5 M), and 0·06 ml sodium periodate solution (0·5% in water, made just before use). One min after addition of the periodate solution, 0·3 ml alkaline sulphite solution (made by dissolving 125 mg anhydrous Na_2SO_3 in 0·5 ml deionized water and adding 4·5 ml of 5 M NaOH, prepared just before use), was added. One min later the following were added as rapidly as possible:

(a) 0·84 ml H_2O, (b) 0·30 ml citrate buffer (0·5 M pH 4·0), (c) 0·51 ml phosphoric acid (Analar).

The sample was then transferred to a quartz cuvette and its relative fluorescence determined spectrofluorometrically at activation 330 nm, emission 390 nm (uncorrected wavelengths). The minimum concentration detectable by this method was of the order of 20 ng ml^{-1} urine, and the actual concentrations found were in the range of 100–300 ng ml^{-1} urine. Determinations of Na^+ and K^+ were made using a Corning 405 flame photometer. In the DA-treated animals DA HCl (Sigma) was administered subcutaneously at 1, 10 and 30 mg kg^{-1} to groups of 6 rats; urine was collected for 1 h and then assayed for sodium and potassium.

RESULTS

In control animals the increase in 24 h urine volume from 6·28 ± s.e.m. 0·7 to 8·5 ± 0·7 ml, due to water-loading, was accompanied by a significant increase in total urinary DA ($P < 0·05$) and potassium excretion ($P < 0·001$), but a decrease in sodium excretion (Table 1).

Similarly, in experiments when 6 h urine collections were made further water-loading (40 ml kg^{-1}) produced a corresponding increase in urine volume and urinary DA (Table 2). However, comparison of the concentration of DA per ml of urine (Table 1 and 2), shows that in both experiments, the concentration of DA fell, suggesting that the increase in total DA excretion after water-loading may be simply a 'wash-out' phenomenon.

In contrast, administration of inorganic salts generally produced an increase both in total DA excreted and in the concentration of DA per ml of urine (Table 1). Only $NaHCO_3$ failed to follow this pattern; a 1% solution increased the concentration of DA, but not enough to produce a significant change in the total amount excreted over 24 h. After a 3% solution, diuresis was accompanied by increased loss of sodium, potassium and dopamine,

Table 1. Effect of water and salt loading (20 ml kg^{-1} p.o.) on 24 h urine composition in the rat. 1% salt loading was given on days 1–3 followed by 3% salt loading on days 4–6. Assays were performed on days 2, 3, 5 and 6.

Salts	Urine vol ml 24 h^{-1}	Dopamine nmol ml^{-1}	Dopamine nmol 24 h^{-1}	Sodium mmol 24 h^{-1}	Potassium mmol 24 h^{-1}
Control non-hydrated	6·28 ± 0·7*	0·97 ± ·008	6·1 ± 0·56*	1·28 ± 0·10	0·07 ± 0·02***
Control hydrated	8·50 ± 0·7	0·88 ± 0·02	7·5 ± 0·02	0·93 ± 0·02	0·22 ± 0·03
NaCl 1%	8·15 ± 0·40	1·19 ± 0·07***	9·7 ± 0·80*	2·20 ± 0·37**	0·17 ± 0·02
NaCl 3%	12·70 ± 0·7***	1·76 ± 0·04***	22·5 ± 1·50***	7·44 ± 0·30***	0·36 ± 0·04***
KCl 1%	9·90 ± 0·7	1·25 ± 0·04***	12·4 ± 1·10***	1·12 ± 0·13	0·50 ± 0·05***
KCl 3%	11·38 ± 0·2***	1·66 ± 0·09***	18·0 ± 0·90***	1·31 ± 0·30	0·96 ± 0·04***
NH_4Cl 1%	9·06 ± 0·4	1·07 ± 0·02***	9·6 ± 0·30***	1·40 ± 0·01*	0·24 ± 0·03
NH_4Cl 3%	12·30 ± 0·4*	1·43 ± 0·03***	17·4 ± 0·70***	2·30 ± 0·08***	0·32 ± 0·16
$CaCl_2$ 1%	11·50 ± 1·2*	0·94 ± 0·02	10·9 ± 1·50*	1·19 ± 0·50	0·17 ± 0·12
$CaCl_2$ 3%	15·30 ± 1·1***	1·19 ± 0·01***	18·4 ± 1·10***	1·49 ± 0·19	0·28 ± 0·03
$NaHCO_3$ 1%	8·10 ± 0·3	0·95 ± 0·01**	7·5 ± 0·02	2·50 ± 0·21***	0·20 ± 0·20
$NaHCO_3$	11·10 ± 0·3**	0·77 ± 0·01***	8·6 ± 0·20***	3·90 ± 0·18***	0·39 ± 0·12**

Means ± s.e.m.; n = 10. $P < 0·05$*, $< 0·01$**, $< 0·001$*** compared with control hydrated group (Student's *t* test).

Table 2. Effects of water loading and diuretics on 6 h urine composition in the rat. Diuretics were given in water in a volume of 20 ml kg^{-1} p.o.

Diuretics	Urine vol ml 6 h^{-1}	Dopamine nmol ml^{-1}	Dopamine nmol 6 h^{-1}	Sodium mmol 6 h^{-1}	Potassium mmol 6 h^{-1}
Control 20 ml kg^{-1} H_2O	5·5 ± 0·5	0·66 ± 0·03	3·7 ± 0·02	0·09 ● 0·02	0·15 ± 0·02
Water loaded 40 ml kg^{-1} H_2O	10·4 ± 1·2**	0·64 ± 0·05	6·2 ± 0·30***	0·09 ± 0·02	0·14 ± 0·09
Frusemide 30 mg kg^{-1}	10·2 ± 0·8***	0·92 ± 0·05***	9·4 ± 0·70***	0·69 ± 0·07***	0·45 ± 0·09**
Frusemide 100 mg kg^{-1}	19·7 ± 1·3***	0·79 ± 0·02**	15·7 ± 1·10***	0·90 ± 0·04***	0·64 ± 0·05***
Hydrochlorothiazide 30 mg kg^{-1}	8·0 ± 0·8*	0·92 + 0·4***	7·4 ± 0·40***	0·44 ± 0·05***	0·37 ± 0·02***
Hydrochlorothiazide 100 mg kg^{-1}	10·4 ± 0·8***	0·87 ± 0·01***	9·4 ± 0·80***	0·43 ± 0·03***	0·42 ± 0·05***
Triamterene 30 mg kg^{-1}	7·6 ± 0·6*	2·15 ± 0·19***	16·4 ± 1·50***	1·12 ± 0·12***	0·41 ± 0·01***
Triamterene 100 mg kg^{-1}	12·7 ± 0·3***	1·43 ± 0·09***	18·2 ± 1·20***	1·69 ± 0·07***	0·34 ± 0·03***

Means s.e.m., n = 10. P <0·05*, <0·01**, <0·001*** compared with control 20 ml kg^{-1} group (Student's t-test).

although the concentration of DA per ml fell significantly.

The most pronounced diuresis was produced by 3% $CaCl_2$ solution, with 3% NaCl producing the greatest increase in DA output. The relationship between urinary DA, and urine volume in salt-treated animals over 24 h showed a good correlation (coefficient r = 0·8 $P < 0·001$) (Fig. 1). In contrast the correlation between excreted DA and sodium was poor (r = 0·47; P <0·1).

Table 2 shows that the expected diuresis and natriuresis following administration of frusemide, hydrochlorthiazide or triamterine was accompanied by significant increases in both the total amount and concentration of DA excreted in the 6 h collection period.

One difference between the effect of the inorganic salts and the diuretics was that after the latter, there was good correlation of DA excreted with the amount of sodium excreted (r = 0·94; P <0·001) (Fig. 2), and, unlike the inorganic salts, there was a lack of correlation between DA excretion and urine volume (r = 0·57, P >0·10).

Chloride concentrations in the urine were not measured but from Table 1 it can be seen that the

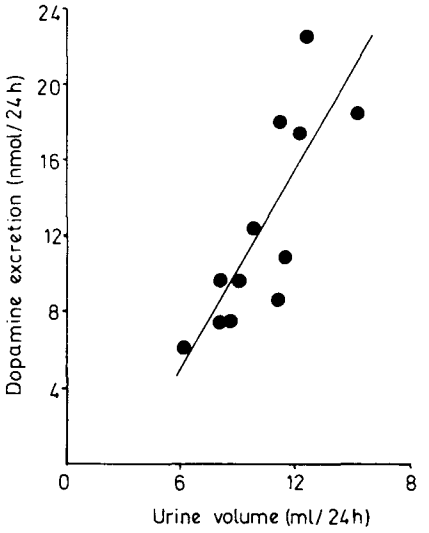

FIG. 1. Correlations between 24 h urine volume and dopamine excretion in rats given 1 and 3% salt solutions 20 ml kg^{-1} p.o.; each point is the mean value for one of the groups in Table 1 (n = 10). r = 0·8, $P < 0·001$.

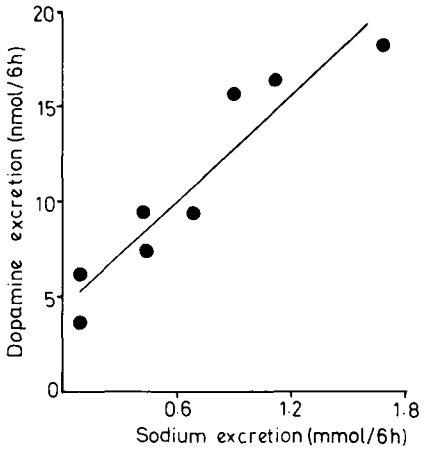

FIG. 2. Correlation between 6 h urinary sodium and dopamine excretion in rats given 30 and 100 mg kg^{-1} p.o. (20 ml kg^{-1}) of frusemide, triamterine, hydrochlorothiazide and water 20 and 40 ml kg^{-1} p.o. Each point is the mean value for one of the groups in Table 2 (n = 10). r = 0·94, $P < 0·001$.

salts that produced the greatest increase in urinary dopamine concentration contained chloride. There was a significant correlation ($P < 0{\cdot}001$) between chloride-loading and dopamine excretion but not between sodium-loading and dopamine excretion ($P > 0{\cdot}05$) (Fig. 3).

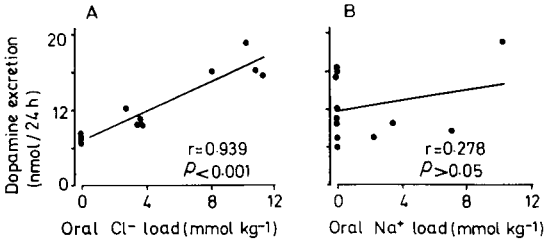

FIG. 3. Correlations between 24 h dopamine excretion and the amount of chloride (A) and sodium (B) administered orally to rats. Each point = 10 rats.

The effect of subcutaneous administration of DA on urine volume and composition is shown in Fig. 4. Both volume and Na^+ concentration were increased up to a maximum at 10 mg kg^{-1} DA, whereas K^+ concentration showed a dose-dependent fall over the same dose range.

DISCUSSION

This study was undertaken to investigate the relationship between DA and urine volume and composition, previously studied extensively only in the dog and man (Alexander et al 1974; Cuche et al 1972).

Of the inorganic salts only 3% sodium bicarbonate failed to produce a significant increase in urinary DA concentration. Our finding of a significant increase in total DA after bicarbonate administration conflicts with that of Ball et al (1978) who reported a decrease. That sodium bicarbonate was the only inorganic salt to increase urine pH, and that oxidation of DA in the urine at alkaline pH may have resulted, might have had a significant effect on the results. Apart from this, results of inorganic salt administration indicate that diuresis and natriuresis are generally associated with an increased DA excretion, and that this loss of DA is not simply a wash-out effect, since the concentration per ml and the total amount of DA excreted is increased, during the period of collection. This is in contrast to the effect of water-loading, which increased total DA output, without increasing the concentration per ml.

Also it is clear that the excretion of DA is not simply or exclusively associated with sodium excretion, which agrees with Ball et al (1978). Inorganic salts containing no sodium produced significant increases in DA excretion, and, DA excretion in animals treated with inorganic salts correlated better with urine volume than with urinary sodium (Fig. 1). A correlation of this kind tends to support the hypothesis of Alexander et al (1974) that an expansion of extracellular volume is involved in increasing DA excretion in urine. Diuretic administration, however, produced an increase in DA excretion that correlated significantly with sodium excretion, rather than with urine volume.

Lastly there was a good correlation between the amount of chloride administered and dopamine excretion (Fig. 3).

Clearly, diuresis induced by a variety of mechanisms, is accompanied by an increase in DA excretion, and it is a possibility that release of DA in the the kidney may play a causative role. That subcutaneously administered DA induces diuresis and natriuresis, adds weight to such a view.

In considering ways in which renal DA might trigger diuresis, it is clear that induction of natriuresis

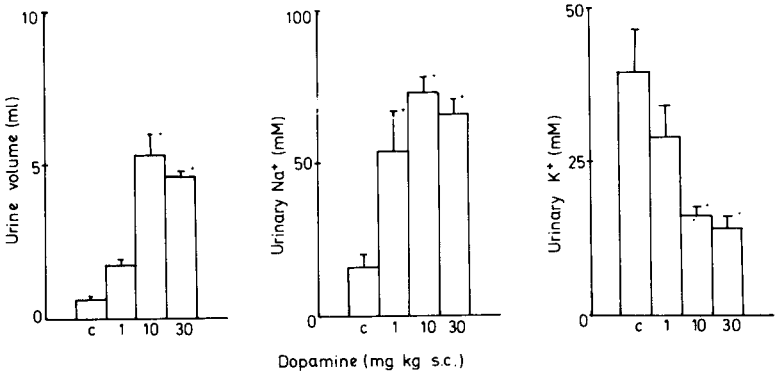

FIG. 4. The effect of dopamine 1–30 mg kg^{-1} s.c. on urine volume and composition. Urine was collected for 1 h. Means ± s.e.m.; n = 6; $*P < 0{\cdot}05$ (Student's *t*-test).

is not a consistent correlate. However, when the effect of the inorganic salts in causing loss of DA is compared with the concentration of chloride ion in the administered dose, there is a significant correlation (Fig. 3). The importance of active chloride transport in the kidney has been established (Kokko 1972; Hogg & Kokko 1979) and it seems therefore, that, if DA contributes to the control of renal function, an action on chloride balance within the kidney should be investigated.

The increased sodium excretion and decreased potassium excretion seen after DA administration indicates that DA might be inhibiting sodium-potassium exchange in the distal tubule possibly by inhibiting the action or production of aldosterone. Evidence in favour of this has been published by Brown et al (1979) who showed that the DA antagonist metoclopramide, caused a three fold increase in plasma aldosterone concentrations while the DA agonist, bromocriptine, lowers plasma aldosterone concentrations in man (Edwards et al 1975).

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