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An investigation of the importance of the adrenal gland to the action of dopamine in the rat kidney

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1 In the rat, administration of dopamine is associated with diuresis, natriuresis and a decreased excretion of K^+. The site of action of dopamine in mediating these responses has been investigated.

2 Urine volume, and urinary Na^+, K^+, Cl^- and dopamine concentrations have been measured in adrenalectomized and sham-operated male Wistar rats.

3 As expected, adrenalectomy decreased urine volume and increased Na^+ and Cl^- excretion; at the same time the amount of dopamine excreted fell, and K^+ excretion did not change.

4 Administration of either 3% NaCl (20 ml/kg orally) or frusemide (100 mg/kg s.c.) significantly elevated dopamine excretion after adrenalectomy.

5 When dopamine, 1, 10 and 30 mg/kg (s.c.) was given to adrenalectomized rats, the diuresis and fall in K^+ excretion seen in control animals was still present. No further natriuresis, over and above the already high urinary Na^+ levels, was observed.

6 The results show that the three actions of dopamine in inducing diuresis and natriuresis, and decreasing K^+ excretion, are clearly separable.

7 It is further argued that in mediating these effects the main site of action of dopamine is the kidney rather than the adrenal gland.

Introduction

There is now considerable evidence that dopamine has a local action on the kidney, and it has been suggested that renal dopamine may play an important role in regulation of blood pressure (Kuchel, Buu, Hamer, Nowacznski & Genest, 1979). Exogenously administered dopamine increases renal blood flow and glomerular filtration rate, and induces natriuresis and diuresis in the dog (MacDonald, Goldberg, McNay & Tuttle, 1964; Goldberg, 1972; Bell, Lang & Laska, 1978); in man (Macgaffey & Jick, 1965) and in the rat (Deis & Alonso, 1970; Akpaffiong, Redfern & Woodward, 1980; Chapman, Horn, Munday & Robertson, 1980). However, controversy surrounds both the exact source and the site of action of dopamine regulating local changes in the kidney. On the one hand it is possible to show that dopamine is synthesized in the kidney (Ball, Oates & Lee, 1978) and that a significant proportion of renal dopamine is located in cortical neurones (Bell *et al.*, 1978; Dinerstein, Henderson, Goldberg & Hoffman, 1978). At the same time an inverse correlation has been noted between plasma renin activity and urinary dopamine (Cuche, Kuchel, Barbeau, Boucher & Genest, 1972) while the dopamine-receptor agonist, bromocriptine, inhibited the increase in plasma aldosterone induced by frusemide without affecting the concomitant increase in plasma renin activity. In addition, a direct action of dopamine on adrenal cells, whereby angiotensin-stimulated aldosterone biosynthesis was consistently inhibited, has also been demonstrated (McKenna, Island, Nicholson & Liddle, 1979) and primary aldosteronism has been correlated with elevated plasma dopamine concentrations and an increased urinary excretion of dopamine (Kuchel *et al.*, 1979). These and similar findings have led to the suggestion that dopaminergic neurones may exert a tonic-inhibitory effect on the production and release of aldosterone (Carey, Thorner & Ortt, 1979). We have previously shown (Akpaffiong *et al.*, 1980) that in the rat, subcutaneously administered dopamine induces both diuresis and natriuresis and

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that when urine composition is altered by administration of a variety of inorganic salts and diuretic agents, diuresis and natriuresis were accompanied by increased dopamine excretion in the urine. In an attempt to elucidate more fully the physiological role of dopamine in the kidney, we have compared dopamine excretion in normal and adrenalectomized rats and have investigated the effect of exogenous dopamine on urine composition after adrenalectomy.

Methods

Male wistar rats (150–160 g) were used in groups of three. Adrenalectomy was performed at about 14 days before use; adrenalectomized animals were subsequently maintained on saline (0.9% w/v NaCl solution). Sham adrenalectomized animals were used as controls. Animals were deprived of food but had free access to fluid for 18 h before being used. During the experimental period animals were deprived of both food and fluid. Prior to the administration of drug, all animals were water-loaded (sham adrenalectomized) or saline-loaded (adrenalectomized) with 20 ml/kg fluid orally. This ensured a constant fluid intake and a sufficient urine output at the end of the collection period for the various analyses. Additionally, changes in urine dopamine release and excretion which might have been caused by difference in food intake were eliminated. Animals were housed in metabolism cages (North Kent Plastics, Kent) which allowed collection of urine free from food and faecal contamination. Urine samples were collected over the same period each day, thus avoiding any differences in free dopamine release and excretion which might otherwise have arisen from circadian or ultradian variations. Dopamine was assayed by a modification of the method of Anton & Sayre (1964), which has been previously described in detail (Akpaffiong *et al.*, 1980). Urinary sodium and potassium were determined in a Corning flame photometer (model 405 Corning, Halstead, Essex). Chloride ion concentration was determined with an Orion selective chloride electrode model 96-17.

Results

The effect of adrenalectomy on urine volume and composition is shown in Table 1 from which it can be seen that in addition to the expected decrease in urine volume and increase in sodium and chloride excretion, there was a fall in the amount of dopamine excreted. However, it should be noted that at the same time the dopamine concentration in the kidney itself had also significantly decreased from 0.7 ± 0.03 mmol/g in normal rats to 0.5 ± 0.006 mmol/g in the adrenalectomized rats. It can also be seen from Table 1 that although the amount of dopamine excreted in the adrenalectomized rat is reduced, the response to either an increase in sodium concentration or to the diuretic agent, frusemide, is still present, suggesting perhaps that although renal tissue levels of dopamine are decreased, the functional pool of dopamine is still available.

We have also investigated the effects of exogenously-administered dopamine on urine composition. Figure 1 compares the effect of dopamine 1, 10 or 30 mg/kg administered subcutaneously in normal and adrenalectomized rats. It will be seen that in the adrenalectomized animals the diuretic response and the fall in potassium excretion seen in control animals are still present but the natriuretic effect has been abolished. However, it must be noted that in the adrenalectomized animal, the basal level of sodium excretion is already raised. In order to investigate the possibility that this high basal level of sodium excretion could have influenced the results in the adrenalectomized animals, the effect of dopamine in

Table 1 Urinary dopamine (DA) excretion in normal and adrenalectomized rats

Treatment		*Urine volume* (ml kg^{-1} 6 h^{+1})	*Na^+ excretion* (mmol kg^{-1} 6 h^{-1})	*K^+ excretion* (mmol kg^{-1} 6 h^{-1})	*Cl^- excretion* (mmol kg^{-1} 6 h^{-1})	*Urinary DA* (nmol kg^{-1} 6 h^{-1})
Control	sham-operated	22.5 ± 1.1	0.2 ± 0.07	0.56 ± 0.04	1.1 ± 0.07	8.3 ± 0.5
($n = 4$ groups of 3)	adrenalectomized	6.4 ± 0.2	1.3 ± 0.05	0.54 ± 0.03	3.2 ± 0.3	1.6 ± 0.05
3% NaCl 20 ml/kg (orally	sham-operated	29.8 ± 1.1***	9.7 ± 0.4***	2.8 ± 0.1***	16.8 ± 0.6***	33.9 ± 1.6***
($n = 12$ groups of 3)	adrenalectomized	27.2 ± 1.3***	10.3 ± 0.6***	2.6 ± 0.2***	14.1 ± 0.9***	9.8 ± 0.6***
Frusemide 100 mg/kg (s.c.)	sham-operated	47.5 ± 0.8***	4.5 ± 0.05***	2.6 ± 0.07***	10.6 ± 0.2***	36.4 ± 0.6***
($n = 10$ groups of 3)	adrenalectomized	72.9 ± 2.3***	8.6 ± 0.5***	3.1 ± 0.2***	15.5 ± 0.5***	36.7 ± 2.3***

Means are shown ± s.e.mean. ***$P < 0.001$ compared to corresponding control value.

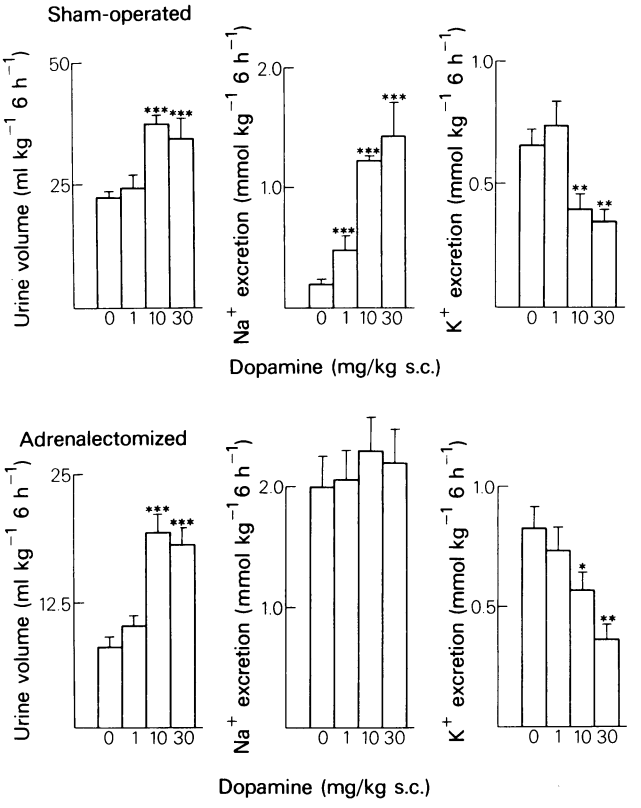

Figure 1 Effect of dopamine on urine composition in adrenalectomized and sham-operated rats. Means of at least 4 groups of 3 rats are shown; s.e.mean indicated by vertical lines. Difference from corresponding control: $*P<0.05$; $**P<0.01$; $***P<0.001$. Dopamine was injected subcutaneously immediately before urine collection began. Adrenalectomized animals were given 20 ml/kg saline orally; sham-operated animals received 20 ml/kg water orally.

adrenalectomized rats has been compared with the effect in normal rats in which basal sodium excretion has been increased by pretreatment with either 3% sodium chloride (orally) or 100 mg/kg frusemide administered subcutaneously. These results are shown in Figure 2 from which it can be seen that in none of these groups of animals was dopamine capable of further elevating sodium excretion. It should also be noted that whereas potassium levels were decreased after dopamine administration in all three groups, only in the adrenalectomized animals was there any diuresis following dopamine administration.

Discussion

In investigating the physiological role of dopamine in the kidney we have asked three questions – first, is the adrenal gland the source of urinary dopamine? Second, where is the site (or sites) of action of dopamine? Third, are the effects of dopamine on urine volume and on sodium and potassium concentrations the result of a single action of dopamine or are they separable?

In considering the first point, the fact that in adrenalectomized animals the levels of excreted dopamine are considerably lower that in control animals would lead one to conclude that the adrenal gland is an important source of urinary dopamine. However, the fact that the response to elevated sodium levels, increased either by administration of sodium chloride or by administration of frusemide, is unaltered (indeed, in some experiments the proportional increase in dopamine excretion was greater in the adrenalectomized animals compared to that in controls) would argue that though the adrenal gland may store dopamine, there is still sufficient dopamine available, possibly in the kidney itself, to mediate the response seen, and that the pool of dopamine in the adrenal gland is of minor importance. It may also be inferred that the slight decrease in renal tissue dopamine observed in adrenalectomized rats repres-

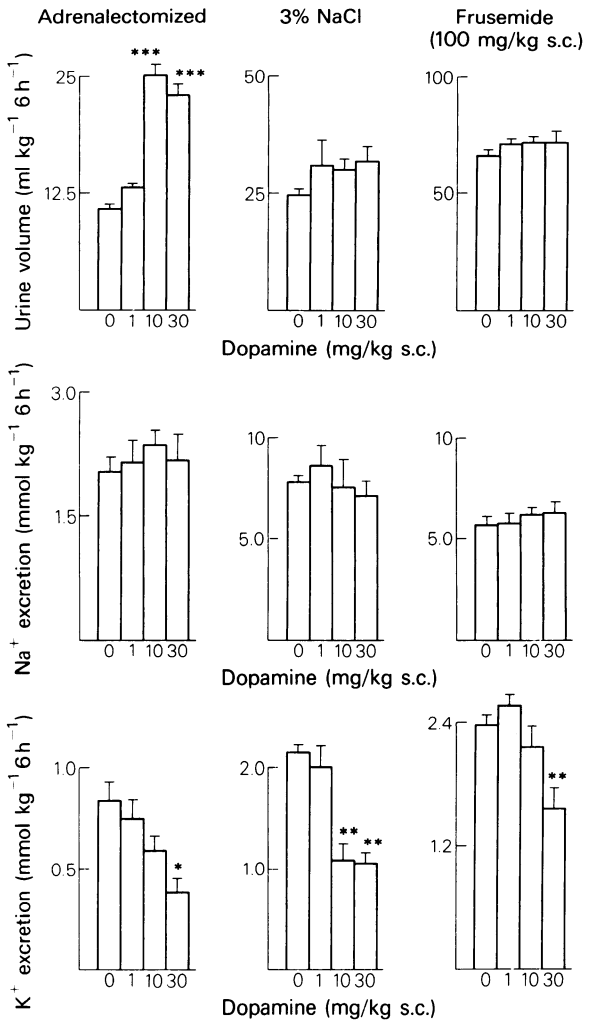

Figure 2 Comparison of the effects of dopamine on urine composition in adrenalectomized rats and in normal rats given either NaCl or frusemide. Means of at least 4 groups of 3 rats are shown; vertical lines indicate s.e.mean. Dopamine was injected (s.c.) immediately before urine collection began. At the same time, normal rats received either 3% NaCl, 20 ml/kg (orally) or frusemide 100 mg/kg (s.c.) and 20 ml/kg water (orally). For levels of significance, see legend to Figure 1.

ents the response to a decrease in plasma electrolyte concentrations.

Turning to the second point, the site of action of dopamine, there is considerable evidence for a direct action of dopamine on the kidney and this has been attributed variously to renal vasodilatation, a direct action on the renal tubule, and inhibition of the action of antidiuretic hormone or aldosterone. Alternatively there is evidence that dopamine may affect renal mechanisms by an action of the adrenal gland; for instance Edward, Mial, Hanker, Thorner & Al-Dujailli, (1975) demonstrated that the specific long-acting dopamine receptor agonist, bromocriptine, inhibited the increase in plasma aldosterone induced by frusemide without altering the concomitant increase in plasma renin activity. Additionally, the dopamine antagonist, metoclopramide, significantly increased plasma aldosterone concentration within 15 min of administration in normal man. Plasma renin activity, free 11-hydroxycorticosteroid and serum potassium concentrations were unchanged by metoclopramide. Administration of dopamine blunted the aldosterone responses to metoclopramide without altering the basal plasma aldosterone

concentrations. In contrast, bromocriptine did not alter basal plasma aldosterone concentrations or the aldosterone responses to metoclopramide (Carey *et al.*, 1979). It was therefore suggested that the aldosterone response to metoclopramide is mediated by antagonist activity at a dopamine receptor site. It has also been demonstrated that dopamine has a direct effect on adrenal cells whereby angiotensin-stimulated aldosterone biosynthesis was consistently inhibited by dopamine (McKenna *et al.*, 1979). Kuchel *et al.* (1979) have shown that patients with primary aldosteronism have elevated free and conjugated plasma dopamine levels and increased urinary excretion of total dopamine compared to control subjects.

The results of our experiments provide no evidence that the adrenal gland is essential to the action of dopamine in regulating renal function. Thus in the adrenalectomized rat the diuretic effect remains unchanged, as does the decreased excretion of potassium. The results of sodium estimations are more equivocal, but there seems no reason to suppose that the failure to increase natriuresis in adrenalectomized animals is not due to the already elevated basal levels of sodium. Therefore, we believe our results lend support to the hypothesis that the action of dopamine in regulating renal function is due to an action on the kidney itself. There is no evidence for an action on the adrenal gland, nor is there any evidence that urinary dopamine is adrenal in origin.

Finally there is some evidence from our results, and from the literature, that dopamine may have more than one action in regulating renal function, and that the effect on urine volume and on the ionic components of urine may be separable. Certainly it is difficult to explain all the actions of dopamine described in this paper by a single mechanism. For instance Deis & Alonso (1970) suggested that the diuresis produced by dopamine could be due to the blockade of vasopressin in the kidney, a view which has been supported by the work of Bentley, (1972) who showed that dopamine competitively inhibited the hydro-osmotic response of the toad urinary bladder to ADH, possibly via an action on adenylate cyclase. However, if inhibition of the effects of ADH were the only, or even the predominant effect in the rat, one would have predicted the production of a dilute urine with roughly proportional changes in sodium and potassium, which is not what we observed. Furthermore there is evidence that several of the manipulations that we employed produced selective changes in one or other of these parameters; for instance, after adrenalectomy, diuresis and a decrease in potassium concentration were still present, whereas dopamine no longer increased sodium excretion. This clearly indicated that an increase in sodium excretion is not a necessary concomitant of the diuretic effect or the decreased excretion of potassium. In the same way, when normal animals were treated either with sodium chloride or with frusemide in order to increase sodium excretion, dopamine failed to exert any diuretic effect, but the decrease in potassium excretion was still present. In this regard it is interesting to note our previous findings (Akpaffiong, Redfern & Woodward, 1981) which showed that the dopamine receptor antagonists, flupenthixol and sulpiride, antagonized the actions of dopamine in causing sodium excretion but were without effect on the diuretic action of dopamine, and appeared to have an additive effect with dopamine on potassium excretion.

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