Circulating dopamine: its effect on the plasma concentrations of catecholamines, renin, angiotensin, aldosterone and vasopressin in the conscious dog

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Summary

1. Six male beagle dogs with carotid loops were infused with sodium chloride solution (150 mmol/l: saline) during control observations followed by dopamine infusion at various rates. Arterial blood samples were drawn during the control period and at the end of each period of dopamine infusion for the measurement of plasma dopamine, noradrenaline, adrenaline, renin, angiotensin II, aldosterone, vasopressin, electrolytes and packed cell volume. Blood pressure and pulse were recorded throughout.

2. The rate of infusion and plasma dopamine levels were closely correlated \( r = 0.99, P < 0.001 \). Plasma dopamine levels two to 20 times basal values produced no significant change in any of the other variables measured; levels 200 times basal values caused a significant increase \( P < 0.05 \) in plasma renin concentration; levels 2000 times basal values were associated with significant increases \( P < 0.05 \) in plasma renin and angiotensin II, packed cell volume and blood pressure, without significant changes in other measurements.

3. Circulating dopamine is unlikely to be important in the control of sodium and water metabolism.

Key words: kidney, plasma dopamine, sodium metabolism.

Introduction

Dopamine is an important neurotransmitter in the central nervous system \[1\]. Its role peripherally, except as a precursor of noradrenaline and adrenaline, is less clear \[2, 3\] and its physiological importance as a humoral agent has yet to be established. Even the presence of dopamine in the circulation of normal subjects has been questioned \[4\]. Much of this ambiguity may now be removed by more reliable methods of measuring plasma dopamine concentration \[5, 6\].

Dopamine may be implicated in salt and water metabolism, possibly through an effect on the renin–angiotensin–aldosterone system \[7\]. Sodium and dopamine excretion are closely related \[7–10\]. Infusion of dopamine in large pharmacological quantities \[11\] or administration of the dopamine agonist, bromocriptine \[2\], increases sodium excretion, whereas inhibition of dopamine formation causes a fall \[9\].

However, in these studies responses have rarely been related to concurrent changes in plasma dopamine and there is little information with which to assess the physiological role of blood-borne dopamine. A series of dogs was therefore infused with dopamine to produce a wide range of plasma concentrations. The levels of plasma dopamine could then be compared directly with changes in circulating levels of renin, angiotensin, aldosterone and vasopressin.

Methods

Six male pedigree beagle dogs, in which the right carotid artery had been exteriorized to facilitate cannulation, were used in the study. The preparation and method of infusion have been described elsewhere \[12\]. For 3 days before the study, the animals were given a diet containing 32
mmol of sodium and 28 mmol of potassium/day with free access to water, but were starved on the morning of infusion.

A 45 min intravenous control infusion of sodium chloride solution (150 mmol/l: saline; 18 ml/h) was followed by dopamine (Intropin; Arner-Stone Laboratories) in the same volume of saline infused consecutively at 0-16, 1-6, 16-0 and 160 mmol min⁻¹ kg⁻¹ (0-03, 0-3, 3-0 and 30 µg min⁻¹ kg⁻¹) each rate being continued for 1 h. Blood pressure was measured throughout by mercury manometer and kymograph. Arterial blood samples were drawn during the control period and just before the completion of each of the four dopamine infusion periods. Total blood loss was less than 120 ml; total infusion volume was 84 ml. Previous work has shown that under these experimental conditions there are no changes in plasma aldosterone and angiotensin II levels [12].

Plasma was separated at 4°C immediately and stored at −20°C until required for analysis. Samples for catecholamine assay were frozen in solid carbon dioxide and stored at −70°C; all were assayed within 2 weeks. Plasma renin concentration [13], angiotensin II [12], vaso-pressin [14] and aldosterone [12] levels were measured by radioimmunoassay and sodium and potassium by flame photometry. Renin values are expressed as µ-units/ml [15]. Plasma dopamine, noradrenaline and adrenaline concentrations were measured by the radioenzymatic method of Da Prada & Zürcher [5], in which the catecholamines are converted into their 3-methoxy derivatives in the presence of crude catechol O-methyltransferase and S-adenosyl-l-[methyl-³H] methionine. The following modifications were included. (1) Plasma was used without deproteinization, but containing ethyleneglycolbis-amino-ethyl ether) tetra-acetate (3 mmol/l). (2) Plasma (100 µl) with and without internal standard was added to incubates containing (in final concentration) Tris (1 mol/l), magnesium chloride (75 mmol/l), benzyloxamine (dopa decarboxylase inhibitor; 0-5 mmol/l), 20 µl of catechol O-methyltransferase solution and 3-6 µCi of S-adenosyl-l-[methyl-³H]methionine (5–15 Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.). The total incubation volume was 150 µl and pH 8-4. (3) The 3-methoxy derivatives were extracted into 1 ml of ether in the presence of tetraphenylboron and back-extracted into 50 µl of hydrochloric acid (0-1 mol/l). This could be applied directly to thin-layer chromatography and avoided the need for freeze-drying. (4) The derivative from dopamine was scraped directly into scintillation vials and counted by using 7 ml of Instagel (Packard).

Noradrenaline and adrenaline derivatives were extracted into 1 ml of ammonium hydroxide (0-05 mol/l) and converted into vanillin by the addition of 50 µl of 4% (w/v) sodium periodate. After 5 min 1 ml of acetic acid (0-1 mol/l) was added and the vanillin extracted into toluene containing 0-6% 5-(biphenyl-4-yl)-2-(4-t-butylphenyl)-l-oxa-3,4-diazole.

The limit of detection calculated as twice the blank value was 0-3 nmol/l for dopamine and 0-1 nmol/l for noradrenaline and adrenaline. The interassay coefficient of variation for dopamine was 12-4%, for noradrenaline 8-6% and adrenaline 11-2%. Each group of samples from an individual dog was analysed in a single assay; each sample was measured in triplicate. Statistical comparisons of the values of each infusion period against mean basal values were made by Wilcoxon's matched-pairs signed-ranks test. Results are expressed as means ± SEM.

Results

Plasma catecholamines during infusion

The mean plasma dopamine concentration during the control period was 0-77 ± 0-14 nmol/l; the basal values of samples taken 20 min (0-78 ± 0-14) and immediately (0-76 ± 0-17) before infusion were similar. Infusion of dopamine caused a significant increase in concentration to 1-4 ± 0-2 nmol/l at the lowest rate, and a very high level (1655 ± 106 nmol/l) was achieved during the 160 nmol min⁻¹ kg⁻¹ infusion (Fig. 1). Plasma concentration and infusion rate were closely correlated (r = 0-99, P < 0-001).

The high levels of circulating dopamine, achieved during infusion, interfered with the assay of adrenaline and noradrenaline. Calculations were made to assess the degree of interference (1 and 8-6% cross-over of counts respectively) to allow correction of noradrenaline and adrenaline values. The disparity between the plasma levels of dopamine and those of noradrenaline and adrenaline made it unrealistic to apply the correction factors at the two highest infusion rates.

The basal plasma noradrenaline levels (0-93 ± 0-12 and 0-89 ± 0-12 nmol/l) were similar and did not change significantly during infusion (1-02 ± 0-18 nmol/l in the first period and 1-06 ± 0-09 nmol/l in the second). Plasma adrenaline levels were 0-60 ± 0-15 and 0-55 ± 0-11 nmol/l in the basal period, 0-78 ± 0-15 nmol/l in the first period and 0-66 ± 0-11 nmol/l in the second. These changes were not significant.
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and 41 ± 9 (160 nmol min⁻¹ kg⁻¹) (Fig. 1). Plasma angiotensin II increased from a mean basal value of 13.4 ± 1.9 to 24.9 ± 3.8 pmol/l in the final infusion period.

In the first hour of infusion (0.16 nmol min⁻¹ kg⁻¹) there was a small but insignificant fall from a mean plasma aldosterone concentration of 180 ± 61 to 130 ± 41 pmol/l (Fig. 1). Five of the dogs showed this decrease in plasma aldosterone, but in one dog the level was unchanged from its basal values. The higher plasma aldosterone levels in the subsequent infusion periods were not significantly different from basal values (Fig. 1).

Mean plasma vasopressin fell slightly during the low rates of infusion from a mean basal value of 1.5 ± 0.3 to 1.3 ± 0.2 pmol/l and 1.2 ± 0.2 pmol/l respectively at the two lower rates of infusion, rising again to 1.8 ± 0.2 and 4.5 ± 1.5 pmol/l respectively at the two higher rates of infusion. These changes did not achieve statistical significance but all dogs except dog no. 6 showed a fall in plasma vasopressin concentration in the second period and all but one dog (dog no. 5) a rise in the final period.

Electrolytes and packed cell volume

Plasma sodium and potassium remained unchanged throughout the study. Packed cell volume changed only during the highest rate of infusion, increasing significantly from 43 ± 1% in the control period to 52 ± 1%.

Behavioural and cardiovascular changes during infusion

The dogs, with the exception of dog no. 6, seemed unaware of the changes in infusion rate until the last hour. During the final hour all the dogs showed varying degrees of restlessness, with frequent licking and salivation; all except dog no. 5 vomited at least once. Dog no. 6 vomited twice during a lower rate of infusion (1.6 nmol h⁻¹ kg⁻¹) and was even more severely affected at the highest rate. On stopping the infusion these ill-effects ceased within a few minutes. Blood pressure was unaffected except at the highest rate of infusion (Fig. 1). During this period it rose markedly in the first few minutes and settled to a mean increase of 27 mmHg. At the end of the period blood pressure was significantly higher (142 ± 4 mmHg) than during the control period (115 ± 7 mmHg). Pulse rate (beats/min) increased in this period (85 ± 2), but was not significantly different from the basal value (74 ± 4).
Discussion

The purpose of our study was to determine whether the effects seen with injected dopamine occur at a concentration of plasma dopamine within a range likely to be encountered under physiological circumstances. The dog was used in this study in preference to man to allow measurement of arterial levels (to which the adrenal gland and kidney are exposed) of the catecholamines and manipulation of plasma dopamine over both physiological and pharmacological ranges.

With the radioenzymatic method described, the basal levels of catecholamines were similar to those reported by others, noradrenaline being present in greater concentration than dopamine and adrenaline [5, 6, 16]. A close correlation was obtained between the infusion rate and plasma concentration of dopamine over a range of more than 2000 times basal levels.

Tjandramaga et al. [17] infused dopamine into anaesthetized dogs at 8, 16 and 32 nmol min⁻¹ kg⁻¹ (1.5, 3 and 6 µg min⁻¹ kg⁻¹) to study its cardiovascular effects. Basal concentrations of dopamine were below the detection limit of the fluorimetric method used, but at rates of dopamine infusion comparable with the higher rates in our study similar plasma levels of dopamine were observed. Although dopamine infusion at high rates (>5 nmol min⁻¹ kg⁻¹) has been described frequently in both man and the anaesthetized dog (for a review see [11]) we are not aware of other previous measurements of plasma levels.

Magnitude of physiological changes in plasma dopamine

Dopamine is present in the plasma of a number of species [6, 16]. In man, plasma levels may approximately double after adoption of the upright posture, exercise or change of sodium status [16, 18, 19]. Handling and restraint of rats may lead to greater increases [16]. Although the physiological range is difficult to define, particularly in the dog, it is likely to be in the range 0.2-5.0 nmol/l. Infusion rates of dopamine of more than 5 nmol min⁻¹ kg⁻¹ result in plasma levels well outside this range.

Plasma noradrenaline and adrenaline

It has been suggested that dopamine might alter sympathetic nerve activity by a presynaptic action [20, 21]. To what extent changes in plasma levels of noradrenaline or adrenaline reflect alterations in this activity is unclear. No demonstrable changes in the circulating levels of noradrenaline or adrenaline occurred even when dopamine levels were 20 times basal values. At high infusion rates noradrenaline and adrenaline levels could not be accurately measured.

Plasma renin

Previous studies of the effect of dopamine administration on plasma renin activity in man or dog are summarized in Table 1 [22-26]. These involved the infusion of large quantities of the compound, ranging from 16 to 64 nmol min⁻¹ kg⁻¹. In our studies plasma levels of dopamine as high as 13 nmol/l produced by infusing at the much lower rate of 1.6 nmol min⁻¹ kg⁻¹ did not alter plasma renin concentration, while a further 10-fold increase in infusion rate and consequently plasma concentration produced only a small increase in renin levels. It seems unlikely, therefore, that circulating dopamine is important in the control of renin release. This does not, of
course, exclude a role for dopamine as a neurotransmitter or local hormone [10, 27, 28]. The mechanism by which dopamine increases renin release at higher rates of infusion (Fig. 1) is uncertain. Dopamine infused at high rates stimulates β-adrenoceptors [11]. Renal β-adrenoceptor stimulation increases renin release and inhibition reduces its release [25]. However, Imbs et al. [26] found that haloperidol, a dopamine antagonist, prevented changes in renin after infusion of the catecholamine, whereas a β-adrenoceptor antagonist did not. In contrast, others [29, 30] observed dopamine-induced renin release was blocked by propranolol, a β-adrenoceptor antagonist, but not by haloperidol.

Although dopamine may have a direct effect on renin release, the increase in plasma renin concentration at the higher rates could simply be in response to the natriuresis induced by dopamine. Sodium excretion was not measured in the present study, but natriuresis has been reported consistently after dopamine infusion at rates exceeding 5 nmol min⁻¹ kg⁻¹ [11].

**Aldosterone**

It has been suggested that dopamine might inhibit aldosterone synthesis or release [31–33]. Administration of metoclopramide, a dopamine antagonist, to human subjects causes a rise in plasma aldosterone concentration without a change in plasma renin activity [31]. Carey et al. [32] claim that dopamine exerts a maximal tonic inhibitory effect on aldosterone secretion which is relieved by antagonists.

Our experiments neither support nor refute this hypothesis. The lowest rate of infusion was associated with a small, but insignificant fall in plasma aldosterone (Fig. 1). At subsequent rates the levels were not significantly different from basal values at a time when renin and angiotensin II levels increased. We cannot rule out the possibility that the absence of a change in aldosterone at high rates is a composite response to the inhibitory effect of dopamine and the stimulating effect of circulating angiotensin II. However, previous studies of the dose–response relationship between aldosterone and angiotensin II in sodium-replete dogs [12] suggest that such modest increases in circulating angiotensin II concentration would produce only small increases in aldosterone levels.

**Vasopressin**

Dopamine may be involved in the control of vasopressin secretion [34]. However, Cadnap-aphornchai et al. [35] could find no evidence of vasopressin release during dopamine infusion, though plasma levels of the peptide were not measured, and Rowe et al. [36] who administered the dopamine agonist, apomorphine, found plasma vasopressin increased only when nausea supervened. Nausea is a potent stimulus to vasopressin release and it may have been responsible for the changes in vasopressin in our studies.

Plasma vasopressin concentration decreased marginally during dopamine infusion at 16 nmol min⁻¹ kg⁻¹ but, as described in the Results section, most of the dogs vomited at the highest rate of infusion and it is probably due to this that the later increases in vasopressin levels occurred. For example, in dog no. 6, the only dog to be affected by vomiting before the final period and the most severely affected at the highest rate of infusion, plasma vasopressin increased to more than 10 pmol/l. In contrast, in dog no. 5, which remained unperturbed and did not vomit even in the final period of infusion, plasma vasopressin changed little throughout the experiment. These findings offer no support for circulating dopamine as an important modulator of plasma vasopressin concentration.

The function of dopamine found in plasma is obscure, but it is unlikely to be that of a circulating hormone concerned with the control of sodium and water metabolism. A role for dopamine as a neurotransmitter outside the central nervous system or as a local regulator of tissue function remains to be elucidated.

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**References**


