Effects of renal dopamine receptor and \( \beta \)-adrenoreceptor blockade on rises in blood angiotensin after haemorrhage, renal ischaemia and frusemide diuresis in the dog

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Summary

1. In chloralose-anaesthetized dogs, central venous and arterial angiotensin (AII) levels were monitored by blood-bathed bioassay during venous haemorrhage of 20 ml/kg, acute renal ischaemia induced by suprarenal aortic stenosis and frusemide-induced diuresis.

2. Blockade of intrarenal dopamine receptors with ergometrine reduced markedly the increments in arterial AII associated with haemorrhage or suprarenal aortic stenosis, but did not consistently affect the corresponding increments in venous AII.

3. Ergometrine or renal denervation did not affect the increases of blood AII associated with frusemide diuresis.

4. Blockade of \( \beta \)-adrenoreceptors with propranolol, by contrast, reduced blood AII increments associated with all three procedures.

5. It is suggested that renin release during moderate haemorrhage and acute suprarenal aortic stenosis is, in the dog, partly due to activation of intrarenal dopaminergic nerves.

6. The possibility is discussed that propranolol may depress renin release in the dog by an action other than that of blocking \( \beta \)-adrenoreceptors.

Key words: \( \beta \)-adrenoreceptor, angiotensin, dopamine, renal nerves, renin.

Abbreviation: AII, angiotensin II.

Introduction

It is now well documented that the renal vascular bed of the dog contains receptors for dopamine which are distinct from \( \beta \)-adrenoreceptors and whose activation causes increased renal blood flow (Eble, 1964; Goldberg, 1972; Bell, Conway & Lang, 1974). We have reported evidence for the existence of intrarenal autonomic nerves which utilize dopamine or a related substance as a transmitter (Bell & Lang, 1973). In view of the evidence that stimulation of the renal nervous supply in the dog produces an increase in plasma renin activity (Vander, 1965; Passo, Assaykeen, Goldfien & Ganong, 1971) it was relevant to determine whether activation of intrarenal dopamine receptors is related to release of renin. In the present series of experiments we have examined this question by investigating the effect of the dopamine-receptor-antagonist ergometrine (Bell et al., 1974; Bell, Conway, Lang & Padanyi, 1975) on the rises in blood angiotensin II (AII) levels associated with moderate haemorrhage, acute suprarenal aortic stenosis and frusemide-induced diuresis. The results obtained have been compared with those obtained after systemic blockade of \( \beta \)-adrenoreceptors by propranolol.

Methods

Surgical preparation and AII assay

Mongrel adult dogs of either sex, weighing between 12 and 20 kg and maintained on a sodium
intake of less than 100 mmol/day, were anaesthetized with intravenous α-chloralose, 70 mg (2.3 × 10⁻⁴ mol)/kg after induction with sodium thiopentone. Supplements of chloralose were administered as necessary during the experiments. All animals were artificially respired under positive pressure and treated with heparin (1000 units/kg intravenously).

Blood pressure was monitored via a polythene catheter passed through the muscular branch of the right femoral artery into the aorta. Mean blood pressure for all dogs studied was 129 mmHg (SEM 2.5, n = 49). Heart rate was monitored from the arterial pressure pulse or from a lead II electrocardiogram. A fine catheter was passed from one femoral artery into the aorta above the renal arteries for administration of drugs into the renal circulation. Arterial blood was sampled from a polythene cannula passed down one common carotid artery into the aortic arch, and central venous blood was sampled from a cannula passed up the right femoral vein into the inferior vena cava just central to the renal veins. Blood was withdrawn from each cannula at a rate of 10 ml/min by using a roller pump (Cole-Parmer Masterflex) and used to superfuse a cascade of isolated bioassay tissues chosen for their specific sensitivities to AI, prostaglandins and catecholamines. The tissues used were rat colon, rat stomach strip and chick rectum (Vane, 1969). At the bottom of the superfusion cascade, both arterial and venous bloods were collected in a reservoir and returned to one external jugular vein by gravity. In order to increase the sensitivity of the isolated rat colon to AI, pronethalol hydrochloride (I.C.I.A.N.Z.), 0.05 mg (2 × 10⁻⁷ mol)/min, was infused into the lumen of the tissue throughout each experiment (Vane, 1969). Under these conditions the increases in blood AI that could be detected, as judged by infusion into the blood superfusing the tissues of pure synthetic AI (Ciba), varied between experiments from 2 × 10⁻¹⁴ to 2 × 10⁻¹³ mol/ml (0.02 to 0.2 ng/ml).

In experiments where diuresis was investigated, both ureters were catheterized via a midline laparotomy before heparinization of the dog. Where the effect of renal denervation was investigated this was accomplished by stripping all visible nerves from the surface of the renal arteries and by wrapping small pledgets of cotton wool soaked in lignocaine (0.09 mol/l) around the arteries. The success of the denervation was supported by a urine flow at least twice that seen under control conditions.

**Stimuli to AI production**

Haemorrhage was accomplished by removal through a side-arm in the jugular venous cannula of 20 ml of blood/kg body weight as rapidly as could be accomplished without allowing diastolic blood pressure to fall below 50 mmHg. This took 2–7 min in different dogs. On completion of the haemorrhage an isovolaemic period of 5 min was allowed, after which the blood was returned to the venous side of the circulation by gravity as rapidly as possible (1–3 min). For production of acute suprarenal aortic stenosis, a cannula bearing an inflatable latex balloon on its tip was passed up one femoral artery to a position in the aorta just central to the renal arteries. The balloon was inflated for periods of 5 min with sodium chloride solution (150 mmol/l) in order to lower aortic pressure distal to its site to a stable value below 40 mmHg. At the end of the inflation period the balloon was deflated over a period of at least 10 s so as to prevent a sudden fall in carotid arterial pressure, which leads to adrenal medullary discharge. Successive periods of haemorrhage or suprarenal aortic stenosis were separated by at least 20 min. Under these conditions the increases in blood AI produced by successive stimuli were reproducible. Neither haemorrhage nor aortic stenosis caused liberation of prostaglandin-like material or adrenaline in amounts detectable by our bioassay system. Frusemide (Lasix, Hoechst) was administered intravenously as a bolus injection of 2 mg (6.6 × 10⁻⁶ mol)/kg. As AI production was studied for only a 20 min period after frusemide no attempt was made to replace urinary water losses accurately. Our blood-bathed bioassay detected only changes in blood hormone levels. We therefore have no information regarding basal circulating concentrations of AI.

Representative changes in blood pressure, heart rate and arterial AI in responses to the three experimental manoeuvres are illustrated in Fig. 1.

**Drugs**

In addition to those drugs mentioned above we used ergometrine maleate (Wellcome), dopamine hydrochloride (Sigma) and propranolol hydrochloride (I.C.I.A.N.Z.). All drugs were prepared in sodium chloride solution (150 mmol/l) and doses cited refer to the above salts. Solutions of dopamine contained 0.2 mg (6 × 10⁻⁷ mol) of ascorbic acid/ml. Ergometrine was administered into the
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Fig. 1. Effects on (a) arterial blood pressure, (b) heart rate and (c) arterial angiotensin II (AII) concentration (rat colon activity) of venous haemorrhage (20 ml/kg), suprarenal aortic stenosis and frusemide, 2 mg (6.6 × 10⁻⁶ mol)/kg intravenously, in anaesthetized dogs. Changes in blood AII were monitored continuously by superfusion of an isolated rat colon (see the Methods section) and quantified by application to the assay tissue of known concentrations of synthetic AII (short horizontal bars: doses in mol/ml). In the experiments shown, the increases approximated to 3 × 10⁻¹⁴ mol/ml (haemorrhage), 5 × 10⁻¹⁴ mol/ml (stenosis) and 4 × 10⁻¹⁴ mol/ml (frusemide).

Renal arterial circulation because this route of administration had always been employed in previous studies, which documented the specificity in the dog vasculature of the drug as a dopamine antagonist (Bell et al., 1974, 1975). Propranolol, on the other hand, was administered intravenously because adequate systemic blockade of vascular β-adrenoceptors was obtained by this route with the dose used and we felt that it minimized any possibility of the non-specific depressant effect of propranolol reported by some workers (Davis & Freeman, 1976). In the doses used ergometrine had no effect on assay tissue sensitivity to AII, but propranolol produced some sensitization.

Statistics

Statistical analysis of mean values was performed with two-tailed Student's t-tests. The tests were paired for analysis of results of haemorrhage and stenosis experiments and unpaired for analysis of results of frusemide experiments.
Results

Haemorrhage

Haemorrhage produced increases in both central venous and arterial levels of AII between $1 \times 10^{-13}$ and $5 \times 10^{-13}$ mol/ml. The mean values obtained were, for arterial blood $2.8 \times 10^{-13}$ mol/ml (SEM 0.5 \times 10^{-13}, n = 12) and for venous blood $2.5 \times 10^{-13}$ mol/ml (SEM 0.4 \times 10^{-13}, n = 9).

After administration of 0.5 mg (1.1 \times 10^{-6} mol) of ergometrine the rise in arterial AII induced by haemorrhage was reduced in seven of eight dogs tested, to a mean value of $1.4 \times 10^{-13}$ mol/ml (SEM 0.2 \times 10^{-13}). This reduction was statistically significant ($P < 0.01$). The effect of ergometrine on haemorrhage-induced increments of venous AII was more variable. In three animals there was reduced production, but in three other animals ergometrine had no effect. The depressant effect of ergometrine persisted for about 60 min. After this period responses similar to those seen under control conditions could be obtained.

Systemic β-adrenoreceptor blockade with propranolol, 0.1 mg (4 \times 10^{-7} mol)/kg, reduced haemorrhage-induced increments in both arterial and venous AII.

Suprarenal aortic stenosis

Acute reduction of renal perfusion pressure below autoregulatory limits produced rises in blood AII of $1 \times 10^{-13} - 7 \times 10^{-13}$ mol/ml. The mean values obtained were, for arterial blood $2.8 \times 10^{-13}$ mol/ml (SEM 0.6 \times 10^{-13}, n = 11) and for central venous blood $2.8 \times 10^{-13}$ mol/ml (SEM 0.4 \times 10^{-13}, n = 11). After intra-arterial injection of ergometrine, 0.5 mg (1.1 \times 10^{-6} mol), the rise in arterial AII induced by stenosis was reduced in six out of seven animals to a mean value of $0.9 \times 10^{-13}$ mol/ml (SEM 0.1 \times 10^{-13}). This reduction was statistically significant ($P < 0.02$). However, as with the haemorrhage experiments, ergometrine had only a variable effect on increments in venous AII, which were unchanged or enhanced in five of eight dogs. Propranolol, 0.1 mg (4 \times 10^{-7} mol)/kg intravenously, caused reduction of ischaemia-induced increments of both arterial (three out of four dogs) and venous AII (two out of three dogs). In the remaining animal propranolol had no effect on either arterial or venous AII.

Frusemide

Intravenous administration of frusemide caused almost immediate diuresis and, as well, detectable increases in both arterial and venous AII levels within 5 min. These reached a plateau within 15–20 min. Mean values obtained were, for arterial AII $3.8 \times 10^{-13}$ mol/ml (SEM 0.7 \times 10^{-13}, n = 6) and for venous blood $2.0 \times 10^{-13}$ mol/ml (SEM 0.4 \times 10^{-13}, n = 6). Over this period no consistent changes in blood pressure or heart rate were observed.

The effect of frusemide on arterial AII levels was compared in six control dogs, in six dogs which received 0.5 mg (1.1 \times 10^{-6} mol) of ergometrine intra-arterially 5 min before frusemide and in six dogs which received 0.1 mg (4 \times 10^{-7} mol) of propranolol/kg intravenously 5–15 min before frusemide. Ergometrine had no appreciable effect on frusemide-induced increments of arterial AII, the mean increase being $3.2 \times 10^{-13}$ mol/ml (SEM 0.6 \times 10^{-13}). By contrast, the increments seen in animals which had received propranolol were in general lower than in control animals, with mean value $1.8 \times 10^{-13}$ mol/ml (SEM 0.5 \times 10^{-13}). This difference was significant ($P < 0.05$). In four dogs the rate of normal urine formation and the diuretic effect of frusemide in left and right kidneys were compared after administration of 0.5 mg (1.1 \times 10^{-6} mol) of ergometrine into the left renal artery only. Ergometrine had no appreciable effect on rate of urine flow under control conditions or on the magnitude of the diuretic effect of frusemide in any of these experiments.

In two dogs with acute bilateral renal denervation frusemide induced rises of arterial AII of $3 \times 10^{-13}$ and $4 \times 10^{-13}$ mol/ml respectively. In three further dogs unilateral renal denervation was performed and the effect of frusemide on venous AII was compared in venous blood from the intact and denervated kidneys by passing separate retrograde sampling cannulae into each renal vein. After frusemide identical increments of AII were seen in the venous effluent from the intact and the denervated kidneys in each experiment. The effect of frusemide appeared therefore to be independent of the renal nerves.

Dopamine infusion into the renal artery

In four dogs dopamine was infused into the renal circulation through a cannula positioned in the aorta just above the renal arteries. Periods of infusion lasting 3 min were employed, at rates of 20 μg (1 \times 10^{-7} mol) min⁻¹ kg⁻¹ (two dogs) and 40 μg (2 \times 10^{-7} mol) min⁻¹ kg⁻¹ (two dogs). Infusion was associated with increments of arterial AII of
Discussion

In the present experiments both haemorrhage and acute suprarenal aortic stenosis were associated with increases of blood All of the order of 2.5 x 10^{-13} mol/ml. Assuming an average cardiac output of approximately 2 litres/min the rate of All production therefore rose by approximately 500 ng (5 x 10^{-18} mol)/min in arterial blood. As there is no reason to assume that either haemorrhage or renal ischaemia of the time course utilized would appreciably alter All metabolism, the changes in blood All observed can be regarded as an index of changes in the rate of renin release.

We observed that intrarenal administration of the dopamine-receptor-antagonist ergometrine reduced the increments in arterial All associated with haemorrhage and with aortic stenosis. In the dose used ergometrine does not depress autonomic transmitter release or axonal conduction (Bell & Lang, 1976), has no antagonist effect towards dilator agonists other than dopamine and does not alter appreciably renal or hind-limb blood flows (Bell et al., 1974, 1975). Furthermore, in the present experiments ergometrine did not affect the rate of urine formation or depress renin release in response to frusemide diuresis. Consequently, its effect cannot be attributed to β-adrenoceptor blockade, to non-selective depression of the release mechanism or to haemodynamic changes.

The depression of renin release in response to haemorrhage or to aortic stenosis seems likely therefore to have been due to the antagonist effect of ergometrine at intrarenal dopamine receptors. In view of the existing evidence for a neural component in the stimulus to renin release in both situations (Bunag, Page & McCubbin, 1966; Hodge, Lowe & Vane, 1966; Ueda, Tagawa, Ishii & Kaneko, 1967; Zanchetti & Stella, 1975) and for the presence of autonomic dopaminergic nerves in the canine kidney (Bell & Lang, 1973), our results are compatible with involvement of these nerves in the process of renin release associated with both haemorrhage and aortic stenosis. Nevertheless, this does not preclude the existence of purely local mechanisms for renin release in response to these stimuli (Blaine & Davis, 1971), activation of which might have been the basis for the residual All increments seen after ergometrine in our experiments.

By contrast, we observed no depression by either ergometrine or renal denervation of the renin release associated with frusemide-induced diuresis, indicating that the mechanism involved here is purely a local one. However, this does not preclude the involvement of the renal nerves in renin release associated with lower doses of frusemide (Naughton, Bertoccello & Skinner, 1975).

It was of interest that the increments of central venous All due to haemorrhage or aortic stenosis were not consistently reduced by ergometrine, implying that some difference might exist between the processes responsible for production of venous and of arterial All. Such a difference would be apparent if ergometrine depressed intrapulmonary conversion of angiotensin I (AI) into All. We have tested this possibility by comparing conversion of 1–2 μg of AI (0.8–1.6 x 10^{-9} mol) during pulmonary passage before and after intra-aortic injection of 1 mg (2.2 x 10^{-6} mol) of ergometrine. No difference was observed in either of two dogs used (C. Bell & W. J. Lang, unpublished observation). There is evidence to indicate that appreciable amounts of All are formed within the kidney before exodus into the renal vein (Thurau, 1974; Mendelsohn, 1976) and this presumably contributed to the venous hormone assayed by our system more than it did to the arterial hormone levels, which would more closely reflect All synthesized within the bloodstream. Another factor which could contribute to the venous All levels monitored is the heptapeptide angiotensin III (AIII), which is formed during the passage of All through the arterial microcirculation (Osborne, d'Auriac, Meyer & Worcel, 1970) and has some biological activity on the rat colon (Notargiacomo & Cohn, 1970). On the other hand, the mechanism by which either of these factors might be differentially affected by ergometrine remains unclear.

It has been demonstrated both in the dog and in other species that isoprenaline stimulates and that propranolol depresses renin release. These data have been interpreted by some workers as implicating an intrarenal β-adrenoceptor in control of renin release. The evidence surrounding this theory
has been reviewed recently by Davis & Freeman (1976). We have confirmed that propranolol depresses renin release due to haemorrhage or to aortic stenosis. In addition we observed that propranolol depressed frusemide-induced renin release, although this release was independent of the renal nerves, as renal denervation itself had no depressant effect.

Despite the widespread assumption that antagonism of a neurally mediated response by propranolol indicates the involvement of postsynaptic β-adrenoreceptors, much of the available evidence regarding propranolol and renin release in the dog suggests that this may not be the case. Propranolol has been reported to antagonize renin release provoked by α-adrenoreceptor agonists as well as by isoprenaline (Chokshi, Yeh & Samet, 1972) and also to antagonize that provoked by cyclic AMP (Winer, Chokshi & Walkenhorst, 1971). Although only the laevo-isomer possesses β-adrenoreceptor antagonist activity, both L-propranolol and D-propranolol have been claimed to be equipotent in depressing isoprenaline-induced renin release (Chokshi et al., 1972). In addition, the observation that propranolol depresses resting renin release from the denervated kidney (Johnson, Davis, Gotshall, Lohmeier, Davis, Braverman & Tempel, 1976) suggests independence of its action from renal nervous influences.

All these data would be compatible with the nervous stimulus to renin secretion being via dopaminergic nerves, and the depressant effect of propranolol being exerted at a level distal to that of the postsynaptic membrane and adenyly cyclase activation. It is of interest that Fuxe, Bolme, Agnati & Everitt (1976) have reported a depressant effect of propranolol on dopaminergic transmission in the rat brain and also that propranolol has potent local anaesthetic effects, suggesting a capacity for depression of basic ionic mechanisms (Mylecharane & Raper, 1973).

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