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The effects of candesartan and ramipril on adrenal catecholamine release in anaesthetized dogs

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Abstract

We have investigated the effects of the angiotensin II type 1 receptor antagonist candesartan, and the angiotensin II converting enzyme inhibitor ramipril, on catecholamine release from the anaesthetized dog's adrenal gland. These drugs were given systemically in low and high doses. The gland was stimulated electrically (0.5–12 Hz) and by angiotensin II infusion (40 ng/kg/min). Electrical stimulation resulted in frequency-dependent increases in catecholamine release. Candesartan (0.8, 4.0 mg/kg) and ramipril (0.125, 0.625 mg/kg) increased basal catecholamine release along with decreases in blood pressure. Both drugs diminished direct nerve stimulation-induced catecholamine release. When both drugs were combined, their inhibitory effect was slightly enhanced. Candesartan blocked catecholamine release induced by angiotensin II. Ramipril was not tested in this respect. The percentage of noradrenaline released during electrical stimulation of the gland remained constant and ranged from 14% to 22%. Both drugs appear to act by blocking local modulation of catecholamine release by the chromaffin cells.

Keywords: Adrenal medulla; Angiotensin; Catecholamine; Canine; Candesartan; Ramipril

1. Introduction

The renin–angiotensin system interacts with the sympathetic nervous system and adrenal medulla to maintain circulatory homeostasis. The main cardiovascular effector of the renin–angiotensin system is the peptide angiotensin II, which regulates peripheral vascular resistance and fluid and electrolyte homeostasis (Goodfriend et al., 1996). Angiotensin II also promotes the release of catecholamines from the adrenal medulla (Foucart et al., 1991).

The renin–angiotensin system is of clinical importance because it is involved in the pathogenesis of several cardiovascular diseases, such as hypertension, arterial disease, diabetic renal disease and heart failure (Gavras and Gavras, 1993). As a result, the class of drug angiotensin converting enzyme inhibitor was developed (Cushman et al., 1977) and successfully used to treat these conditions (Garg and Yusuf, 1995; Chalmers, 1999). Recently, a new and more specific class of renin–angiotensin system antagonist has been developed that act directly at the angiotensin II receptor site (Timmermans et al., 1993). The circulatory and renal effects of these drugs have been well documented (Goodfriend et al., 1996; Timmermans et al., 1993; Carr and Prisant, 1996; Pitt et al., 1997). However, their effects on other functions of the renin–angiotensin system are less well described and understood.

The in vivo effects of the angiotensin converting enzyme inhibitors (enapril and captopril) on the adrenal medulla were first investigated by Critchley et al. (1988) and MacLean and Ungar (1986) who showed that they inhibited catecholamine release during splanchnic nerve stimulation of the dog adrenal gland. Martineau et al. (1995, 1999) have investigated the effects of a number of angiotensin II type 1 antagonists on angiotensin II-induced catecholamine release from the dog adrenal gland. These studies showed that the angiotensin II receptor played an important role in local

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regulation of catecholamine release from the adrenal chromaffin cells and that this effect could be blocked by the angiotensin II type 1 receptor anatgonists. However, these two classes of drugs have not been compared in respect to their effects on adrenal catecholamine release. Thus, we have compared the in vivo effects on electrically stimulated adrenal catecholamine release of an angiotensin II type 1 receptor antagonist, candesartan, and an angiotensin converting enzyme inhibitor, ramipril, using an anaesthetized dog model.

2. Materials and methods

2.1. Anaesthesia and maintenance of homeostasis

Ethical approval for the study was obtained from the Animal Research Ethics Committee of the Chinese University of Hong Kong and the study adhered to Australian and North American guidelines for animal research (see *The Australian code of practice for the care and use of animals for scientific purposes*, 1997. 6th ed. Canberra, Australia. and *The National Health and Medical Research Council*, 1996. 1st ed. Washington, USA). Male mongrel dogs, weighing 15–25 kg, were provided by the Laboratory Animals Service Centre of the Chinese University of Hong Kong. Anaesthesia was induced using intramuscular ketamine 10% (5 mg/kg) and xylazine 2% (2 mg/kg) and maintained throughout the experiment using an infusion of sodium pentobarbital 20% (3–4 mg/kg/h). The trachea was intubated and the dog was allowed to breathe spontaneously on air supplemented with oxygen (1–2 l/min). The right femoral artery and vein were cannulated. Arterial pressure was monitored by a pressure transducer connected to a heated wire on paper recorder. The venous access was used to administer intravenous fluids (warmed saline at 1 ml/kg/h) and drugs, as well as the measurement of central venous pressure. Regular blood gas analysis was performed. Hypoxia was prevented by supplementary oxygen and pH maintained within the range 7.35–7.45 by respiratory adjustment via titration of sodium pentobarbital. Body temperature was maintained near 37 °C by use of a water-heated operating table and covering the dog with an insulated blanket.

2.2. Denervation and electrical stimulation of the left adrenal gland

The left adrenal gland was exposed via a subcostal incision. The left greater splanchnic nerve to the gland was identified (by observing a small blood pressure rise to electrical stimulation) and dissected free. An arc of tissue was crushed medial and rostal to the nerve to eliminate adrenal sympathetic fibres not carried by the nerve. The nerve was firmly crushed (but not sectioned) and ligated with silk as far from the gland as possible, to prevent retrograde nerve conduction. Bipolar platinum electrodes were applied to the nerve. The nerve and electrodes were kept moist by covering them with wet gauze. The nerve was electrically stimulated for periods of 1 min using a Grass student stimulator model SD–9D (Grass Instruments Medical, Quincy, MA, USA). Electrical pulses of 10 V and 2-ms duration were used.

2.3. Collection and preparations of adrenal venous blood

The left adrenolumbar vein was identified and canulated with silicone tubing. The vein was loosely ligated with a silk sling at the junction with the inferior vena cava. Gentle tension on the sling temporarily blocked the venous drainage from the gland and caused blood to flow via the silicon tubing into a collection tube. The dog was anti-coagulated with heparin 500 i.u./kg/h. Blood samples (3.5 to 4.0 ml) were collected from the adrenal vein into pre-chilled conical tubes containing 50 μl of 25% $Na_2S_2O_3$ solution and stored in ice. The volume of blood and time taken for collection were recorded. The samples were later centrifuged at 3000 rpm at 4 °C for 15 min and the blood and plasma volumes were measured. The separated plasma was stored at − 70 °C for subsequent catecholamine assay by high performance liquid chromatography using a modified alumina absorption method, mobile phase, column and coulometric detector.

2.3.1. High performance liquid chromatography—reagents

Alumina, Tris base, DHBA, EDTA, octanesulfonic acid, adrenaline barbiturate, noradenaline bitartrate (Sigma, St Louis. MO, USA), citric acid, sodium acetate (Merck, Darmstadt, Germany), Milli-Q water from water purifier (Millipore, Bedford, MA, USA), high performance liquid chromatography grade methanol (BDH, Poole, England).

2.3.2. High performance liquid chromatography—extraction

The catecholamines were extracted from plasma by modified alumina adsorption method, which is described. To each 50 μl of plasma was added 0.2 ml of 3,4-Dihydroxybenzylamine-Hydrobromide (DHBA) the internal standard (50 ng/ml), 50–60 mg of oxidized alumina and 3 ml of 1 M Tris base, to make Tris buffer. The mixture was tumbled for 10 min and centrifuged for 1 min at 3000 rpm at 4 °C and supernatants discarded. The remaining alumina was washed three times with 2 ml of Milli-Q water (pH 7–8) and the washings discarded. After the final wash, the mixture was again centrifuged for 1 min at 3000 rpm at 4 °C to recover as much alumina and discard as much water as possible. Then 0.75 ml of 0.5 M acetic acid was added to the alumina. The mixture was vortex-mixed for 1 min and centrifuged for 1 min at 3000 rpm at 4 °C. The acid extract was transferred into a 1.5 ml Eppendorf microtube, the top was covered with parafilm with a couple of perforations, frozen and then freeze-dried overnight. The lyophilized sample was prepared for HPLC by reconstitution with 0.2 ml of a mobile phase.

2.3.3. High performance liquid chromatography—mobile phase

A mixture of 13%(v/v) methanol and 87% water (0.02 M citric acid, 0.1 M sodium acetate, 2 mM EDTA and 29 mM octanesulfonic acid) was prepared. The pH of this mixture was 4.7 to 4.8. It was filtered through a 0.22-μm filter and degassed in an ultrasonic bath for 10–15 min before use. The eluent was delivered into the column at a flow-rate of 1 ml/min. The injection volume varied from 10 to 70 μl.

2.3.4. High performance liquid chromatography—analyser

A Waters M510 isocratic pump and 717-plus automated sampling injector (Waters, Milford, Massachusetts, USA). An Ultrasphere IP-C_{18} column (25 cm × 4.6 μm) (Bechman, Gagny, France). A dual-cell Coulochem M5100A detector equipped with a guard cell and an analytical cell, both cells of which were proceeded by a carbon in-line prefilter (Environmental Science Associates, MA, USA). An HP 3396A integrator (Hewlett Packard, USA). The coulometric detector was used with the guard cell set at +0.40 V and the analytical cell set at +0.30 V. The noradrenaline and adrenaline content were measured using an internal standard (DHBA), with regular quality control. The limits of detection were noradrenaline 0.3 nM and adrenaline 0.3 nM.

2.4. Stimulation of the adrenal gland with angiotensin II

Angiotensin II was prepared as a 4-μg/ml solution by mixing the raw peptide (Sigma) with normal saline. It was administered intravenously into the femoral vein using a Terufusion syringe pump model STC-521 (Terumo, Tokyo, Japan) at a rate of 40 ng/kg/min. A pilot study had shown that this rate increased systolic arterial blood pressure by 25–30 mm Hg. The onset of action of the angiotensin was rapid, within 30 to 60 s, and it was infused over 5 min during which time the adrenal blood samples were collected. Following the final dose of candesartan (see below), blood samples were also collected during a higher infusion rate of 400 ng/kg/min.

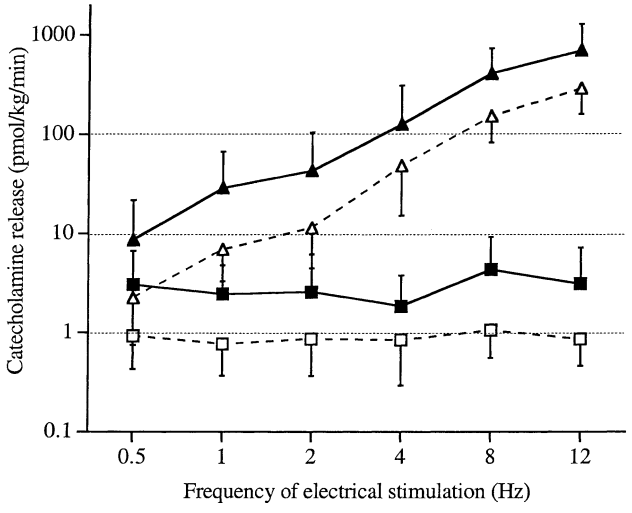

Fig. 1. Basal and electrically stimulated catecholamine releases (mean (S.D.)) from the dog adrenal gland ($n=12$). A logarithmic scale is used. Broken line = noradrenaline; continuous line = adrenaline; squares (□, ■) = basal release; and triangles (△, ▲) = electrical stimulation.

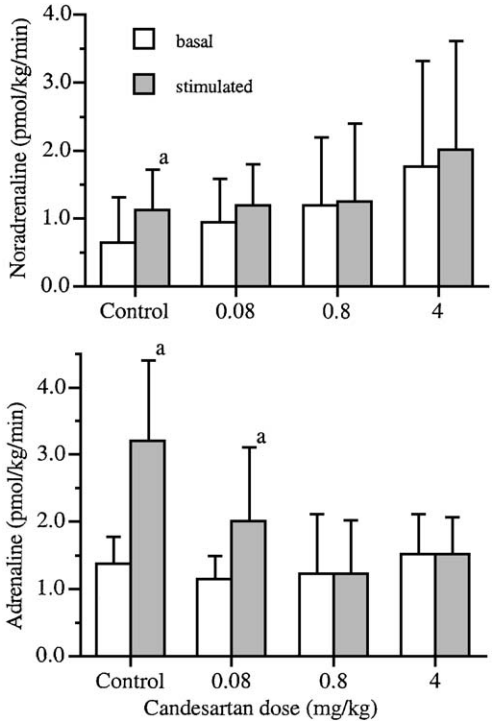

Fig. 2. Noradrenaline (upper) and adrenaline (lower) basal and angiotensin II simulated releases (mean (S.D.)) from the adrenal medulla ($n=5$) as the dose of candesartan was increased. Significant difference compared to basal value shown by $^{a}P<0.05$.

2.5. Preparation of study drugs

Pure injectable formulations were not readily available, so solutions of candesartan and ramipril for intravenous use were prepared from commercially available tablets, which were crushed and ground into a fine powder. The powder was mixed with 5 ml of normal saline per tablet and sonicated for 1 h to dissolve the drug. The resulting solution was then filtered through a 0.4-μm membrane before use.

2.6. Plan of research

The effects of electrical stimulation of the left adrenal gland, at increasing frequencies of 0.5, 1, 2, 4, 8 and 12 Hz, were demonstrated in 12 anaesthetized dogs. The gland was allowed to recover for 10 min following the 0.5- and 1-Hz stimulations, for 20 min following the 2- and 4-Hz stimulations and for 30 min following the 8- and 12-Hz stimulations. Adrenal vein blood samples were collected for catecholamine assay immediately before and during each of the stimulations. The amount of blood and the time for collection were recorded in order to calculate the rate of catecholamine release.

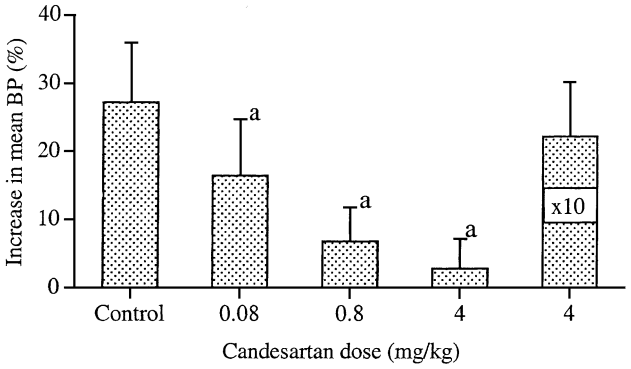

Fig. 3. Increases (mean (S.D.)) in mean arterial blood pressure (%) in response to infused angiotensin II as the dose of candesartan was increased. The right hand column shows the effect of increasing the angiotensin II infusion rate by 10. Significant difference compared to control values shown by $^{a}P < 0.05$.

The effects of three doses of candesartan (0.08, 0.8 and 4.0 mg/kg) on angiotensin II-induced catecholamine release was demonstrated in five anaesthetized dogs. There was a wait of 45 min for the pharmacological effects of candesartan to become established after each dose. Angiotension II (40 ng/kg/min) was then infused systemically over 5 min. Adrenal vein blood samples were collected for catecholamine assay immediately before and during each infusion. After the final set of blood samples had been collected, a further higher concentration of angiotensin II (400 ng/kg/min) was infused and another set of blood samples collected.

The effects of systemically administered candesartan and ramipril on electrically stimulated catecholamine release were tested in anaesthetized dogs. Eight received candesartan, six ramipril and four a combination, or mixture, of both drugs. A low dose followed by a high dose was given. For candesartan, doses of 0.8 and 4.0 mg/kg were used and for ramipril, 0.125 and 0.625 mg/kg were used. The gland was electrically stimulated using frequencies of 0.5, 1, 2 and 4 Hz. Stimulations were performed prior (baseline) and 45 min after each drug administration. The gland was allowed to recover for 7 min following the 0.5- and 1-Hz stimulations and 15 min following the 2- and 4-Hz stimulations. Adrenal vein blood samples were collected during each of the stimulations.

2.7. Drugs

Candesartan cilexetil (Blopress 16 mg/tablet, Takeda Chemical Industries, Japan) and ramipril (Tritace 2.5 mg tablet, Hoechst, Germany).

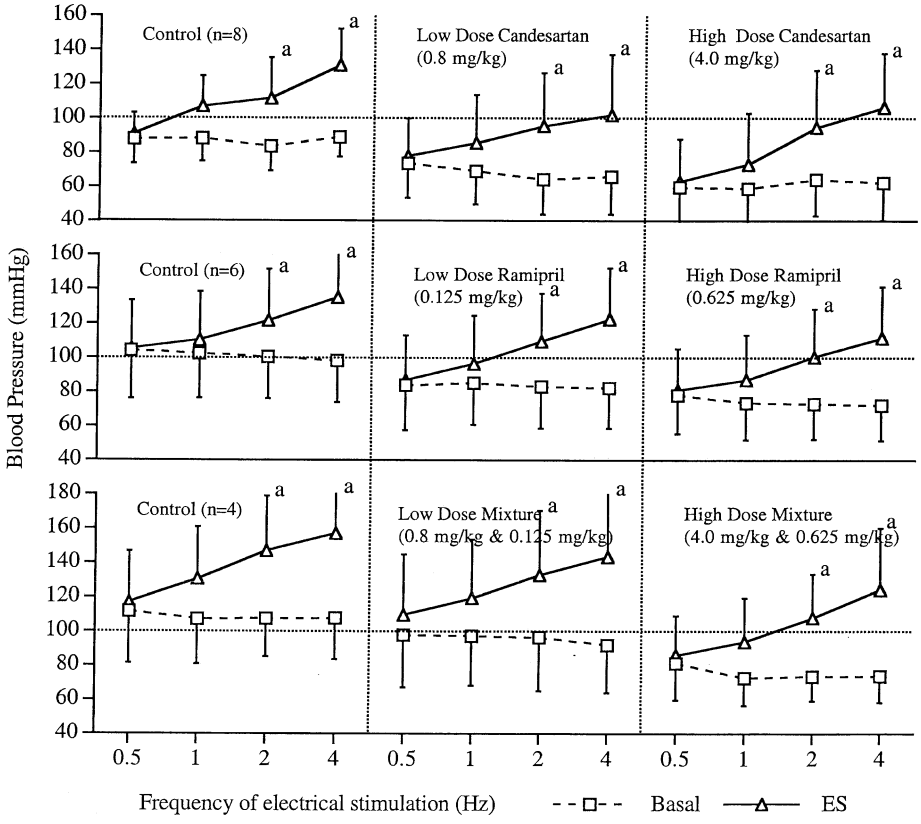

Fig. 4. Mean arterial blood pressure (mm Hg) measurements (mean (S.D.)) during electrical stimulations (ES) of the gland following administration of candesartan (upper), ramipril (middle) and the mixture (lower). Measurements taken at rest (broken line or □) and during ES (solid line or Δ) are shown. Control measurements (left) are compared with those following low dose (centre) and high dose (right) administrations. Significant difference compared to resting values shown by $^{a}P < 0.05$.

2.8. Measured parameters

Blood pressure changes were assessed using the mean arterial blood pressure. The percentage decreases compared to resting pressures were used to assess the effects of drug treatment on blood pressure. The average decrease over the range of frequencies tested was used.

Catecholamines were measured by high performance liquid chromatography in moles (pmol) and later standardized to the weight of each animal (kg) and rate of blood collection (min). Data for both noradrenaline and adrenaline release were presented. The percentages of noradrenaline of the total catecholamine release were also calculated. The percentage increases or decreases compared to basal values were calculated, where basal referred to the non-stimulated catecholamine release. The total catecholamine release was used to make comparisons between the control group and electrically stimulated releases, when drug treatments were investigated.

2.9. Statistical methods

The trends in mean arterial blood pressure measurements and catecholamine releases, as the frequency of electrical stimulation was increased, were compared using analysis of variance for repeated measures (ANOVA-RM). Paired *t*-tests were used to make comparisons between individual sets of data. Data were presented as mean (standard deviation). $P < 0.05$ was considered statistically significant.

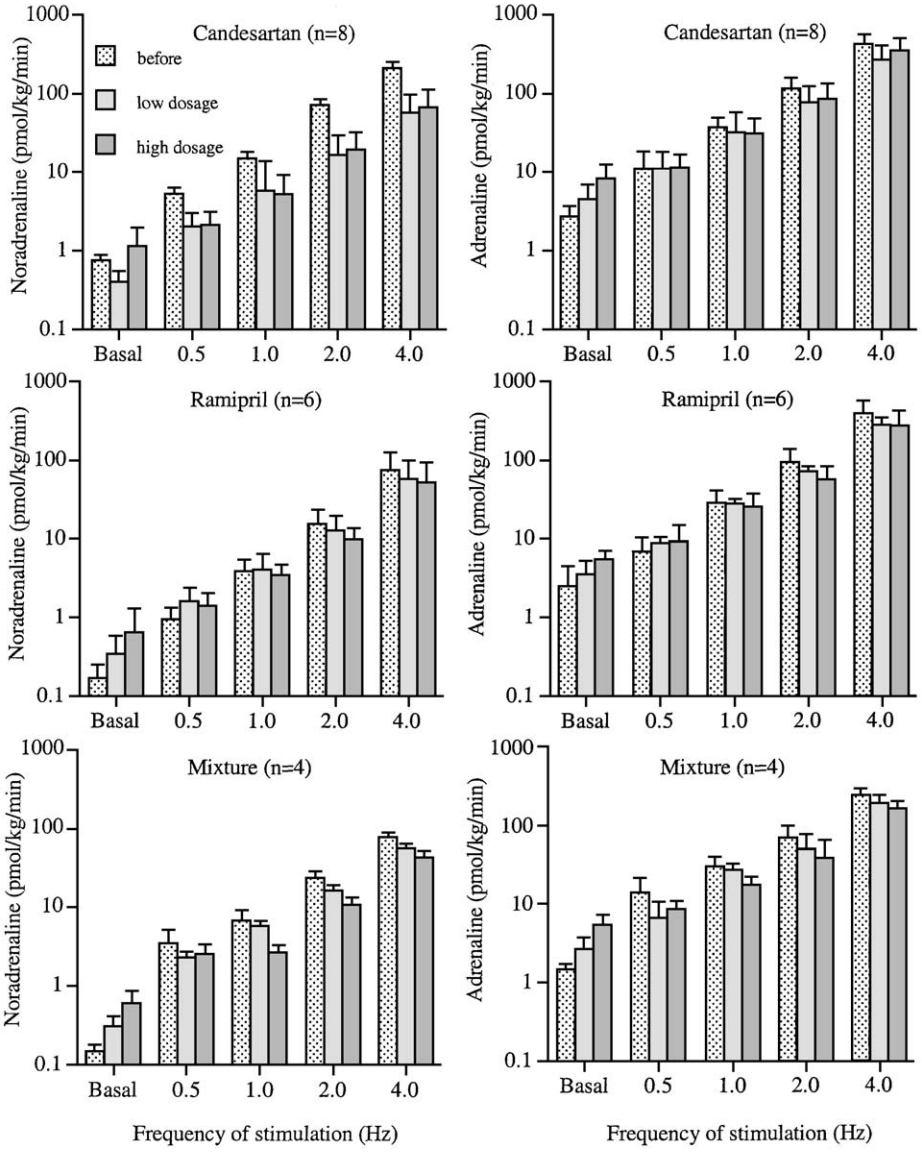

Fig. 5. The effects of candesartan (upper), ramipril (middle) and the mixture (lower) on catecholamine release (mean (S.D.)) in response to electrical stimulation (basal to 4 Hz) of the dog adrenal gland. Logarithmic scale used. Data for noradrenaline (left) and adrenaline (right), before (control) and after administration of the low and high doses, are shown.

3. Results

3.1. Electrically stimulated catecholamine release

The basal release of catecholamines between stimulations remained constant over time as frequency was increased from 0.5 to 12 Hz. When the gland was electrically stimulated, the catecholamine release increased in a non-linear frequency-dependent pattern, which was most simply presented using a logarithmic scale (Fig. 1).

3.2. Angiotensin II-induced catecholamine release

The effect on catecholamine release of the angiotensin II infusion, before candesartan was given, was to increased noradrenaline release by 77 (26)% and adrenaline release by 134 (19)% ($P<0.05$). Candesartan in doses of 0.8 mg/kg and above abolished this enhancement of catecholamine release by angiotensin II (Fig. 2).

3.3. Blood pressure changes

Mean arterial blood pressure was increased by 27 (9)% during the angiotensin II infusion. There were no accompanying changes in heart rate or central venous pressure. Candesartan inhibited the pressor effect of angiotensin II dose-dependently. The inhibitory effect of candesartan was reversed by a ten-fold increase in the infusion rate of angiotensin II (Fig. 3).

Electrical stimulation of the gland produced frequency-dependant increases in the mean arterial blood pressure ($P<0.01$: ANOVA-RM) (Fig. 4). The effect of candesartan was to reduce the overall blood pressure by 22 (3)% for the low dose and 27 (7)% for the high dose. Similarly, ramipril decreased the blood pressure by 22 (4)% and 28 (3)%, respectively, and the mixture decreased the blood pressure by 15 (2)% and 24 (3)%, respectively (Fig. 4).

Table 1
Percentage increases or decreases (mean (S.D.)), compared to control values, for basal and electrically stimulated (0.5 to 4 Hz) total (noradrenaline plus adrenaline) catecholamine releases for both the low and high doses of each drug treatment

	Basal	0.5 Hz	1 Hz	2 Hz	4 Hz
Low dose					
Candesartan	72	−2	−20	−37	−39
(n=8)	(68)%[a]	(28)%	(61)%	(28)%[a]	(32)%[a]
Ramipril	16	26	−11	−20	−32
(n=6)	(45)%	(67)%	(33)%	(12)%[a]	(12)%[a]
Mixture	82	−40	−10	−28	−26
(n=4)	(8)%[a]	(23)%	(80)%	(13)%[a]	(15)%[a]
High dose					
Candesartan	227	−9	−16	−30	−16
(n=8)	(161)%[a]	(30)%	(39)%	(21)%[a]	(23)%[a]
Ramipril	53	0	−18	−29	−31
(n=6)	(36)%[a]	(32)%	(44)%	(24)%[a]	(30)%[a]
Mixture	264	−24	−30	−48	−40
(n=4)	(126)%[a]	(42)%	(58)%	(6)%[a]	(11)%[a]

Significant differences compared to control values shown by [a]$P<0.05$.

Table 2
Percentages of noradrenaline released (mean (S.D.)) from the left dog adrenal gland at different frequencies (basal to 4 Hz) of electrical stimulation

	Basal	0.5 Hz	1 Hz	2 Hz	4 Hz
Controls (n=18)	9 (6)%	16 (5)%	14 (5)%	17 (5)%	18 (6)%
Candesartan (n=8)	10 (6)%	17 (6)%	14 (5)%	19 (6)%	17 (6)%
Ramipril (n=6)	7 (3)%	15 (6)%	14 (6)%	15 (6)%	15 (6)%
Mixture (n=4)	10 (2)%	22 (5)%	15 (4)%	22 (4)%	21 (2)%

Data for controls (no drug treatment) and following administration of candesartan, ramipril and the mixture are shown. Data from both the low and high dose administrations have been combined and their average taken.

3.4. Drug administration on electrically stimulated catecholamine release

There was an overall trend towards higher basal levels and lower stimulated levels of noradrenaline and adrenaline release when treatment with the higher doses of candesartan, ramipril and the mixture was given ($P<0.05$: ANOVA-RM). There was no demonstrable difference between the three drug treatments in their effects on catecholamine release (Fig. 5). The percentage changes in the total catecholamine release during the stimulations, when compared to control releases, are summarized in Table 1. Significant differences compared to control releases were seen with the basal and 2- and 4-Hz stimulated releases ($P<0.05$).

3.5. Selective catecholamine release

Electrical stimulation of the gland (0.5 to 12 Hz) when no drug treatment was given resulted in consistent percentages of noradrenaline of 15% to 25% being released. Electrical stimulation of the gland (0.5–4 Hz) in association with drug treatment also resulted in consistent percentages of noradrenaline being released. Basal percentages were 7% to 10% and these increased to 14% to 22% with stimulation (Table 2).

Candesartan increased the percentage of noradrenaline released during stimulation by angiotensin II. As the candesartan dose was increased, the basal percentage increased from 31 (13)% (control) to 54 (6)% (4 mg/kg) and the stimulated (angiotensin II) percentage increased from 27 (7)% (control) to 57 (5)% (4 mg/kg) (Fig. 2).

4. Discussion

Our study showed that systemically administered candesartan and ramipril increased basal catecholamine release along with decreases in the mean arterial blood pressure. Both drugs diminished direct nerve stimulation-induced catecholamine release. When both drugs were combined, their inhibitory effect was enhanced. Candesartan blocked

angiotensin II-induced catecholamine release. Ramipril was not tested in this respect. Catecholamines were released non-selectively during electrical stimulation. The percentage of noradrenaline released ranged from 14% to 25%.

There was a frequency-dependant relationship between electrical stimulations and the release of catecholamines by the gland, which increased nonlinearly over the range of frequencies tested and was presented using a logarithmic scale (Figs. 1 and 5). If a wider range of frequencies had been tested, then it is probable that the response curve would have been sigmoid in shape because a maximal response would be reached. To achieve maximal catecholamine release from the mammalian adrenal gland, it needs to be electrically stimulated at 15–40 Hz (Edwards et al., 1980; Foucart et al., 1987). In the present study, we used frequencies of up to 12 Hz. Foucart et al. (1987) have shown in dogs that electrical stimuli of 1, 3 and 10 Hz resulted in maximal catecholamine release being obtained almost immediately, which were sustained for at least 10 min. Greater stimuli of 25 Hz did not produce a sustained release (Foucart et al., 1987). Thus, electrical stimuli of 1 and 3 Hz have been mostly used in animal studies (Foucart et al., 1988, 1991; Hosokawa et al., 2000; Kimura et al., 1992; Koji et al., 2003). In the present study, we used a greater number and range of frequencies (0.5, 1, 2 and 4 Hz), which gave a fuller picture of drugs effects (Fig. 5).

Recently published experimental work where the adrenal gland has been pharmaceutically stimulated has involved the direct administration of agents into the gland via the adrenal artery (Martineau et al., 1995, 1996, 1999; Yamaguchi et al., 1999). In our study, angiotensin II was administered systemically via the femoral vein, which resulted in relatively small two to three-fold increases in catecholamine release, despite significant circulatory effects. In comparison, direct stimulation of the gland resulted in much greater increases (Figs. 1 and 5). Furthermore, we did not measure the systemic levels of circulating catecholamines, even though in hindsight, they may have contributed to the total catecholamine content of the adrenal blood samples. Data from Yamaguchi et al. (1999) suggest that adrenal blood levels of basal noradrenaline release are 10 times and adrenaline release are 100 times greater than their systemic circulation counterparts. Thus, any systemic contribution during direct electrical stimulation of the gland would be of little consequence, as adrenal catecholamine release is magnitudes greater than the circulating level. However, the circulatory contribution may have become significant when angiotensin II was infused, as adrenal catecholamine release was not greatly elevated while circulating levels were raised. Hence, the candesartan–angiotensin II data from our study may have reflected circulatory rather than adrenal gland events, making this data somewhat unreliable. The main reason for using angiotensin II in the study was to demonstrate that the intravenous preparation of candesartan was pharmacologically active. However, as ramipril acted via a different pathway, blocking the conversion of angiotensin, the use of angiotension II to test the activity of the preparation of ramipril was inappropriate.

Candesartan and ramipril were found to increase basal catecholamine release (Table 1). In those studies where direct drug administration into the adrenal gland has been used, no increase in basal catecholamine release has been reported (Yamaguchi et al., 1999). Thus, the origin of these increases would appear to be related to the systemic administration of candesartan and ramipril. The systemic administration of both candesartan and ramipril resulted in quite significant decreases in blood pressure of 24% to 28% (Fig. 4). These blood pressure decreases would have been expected to cause some degree of sympathetic response with accompanying release of systemic catecholamines, predominantly noradrenaline from the sympathetic nerve endings. Thus, circulating catecholamines could have reached levels high enough to influence the adrenal vein collection. However, this explanation seems unlikely as there was no accompanying increase in the percentage of noradrenaline collected (Table 1). Another possibility is that some of the sympathetic nerve fibres to the left adrenal gland may have remained intact, despite our best efforts to block nerve conduction. Thus, the gland may have been stimulated during the hypotension by these intact fibres and this may account for the rise in basal catecholamine release during drug treatment. However, the increase in catecholamine release was comparatively small compared to that seen during electrical stimulation, suggesting that only a small number of nerve fibres were involved. The renin–angiotensin system may also have played a part. Hypotension due to hypovolaemia from haemorrahage can cause a marked renin–angiotensin system response and angiotensin II release. It is possible that the resulting angiotensin II stimulates the chromaffin cells to release more catecholamine (Kimura et al., 1992). Martineau et al. (1995, 1999) have recently described the presence of angiotensin II receptors on the chromaffin cell and these receptors are thought to modulate the release of catecholamines. However, this explanation would seem unlikely because candesartan and ramipril block the action of the renin–angiotensin system. Thus, the most likely explanation for the increase in basal catecholamine release following candesartan and ramipril administration would be that drug induced systemic hypotension caused increased sympathetic activity and the adrenal gland was stimulated by nerve fibre conduction that we failed to block by crushing the left greater splanchnic nerve.

During direct electrical stimulation of the gland, both candesartan and ramipril reduced catecholamine release. However, compared to basal release, the catecholamine release during direct nerve stimulation was many times greater. Thus, circulating levels of catecholamines play a much less important role. Yamaguchi et al. (1999) have suggested that during direct stimulation of the gland angiotensin II and other elements of the renin–angiotensin system are released that may play a role in local regulation of

catecholamine secretion. Hence, candesartan may act locally by inhibiting this regulation by blocking the angiotensin receptor on the chromaffin cell, while ramipril prevents local conversion of angiotensin to its active form. Thus, both drugs may act by inhibiting stimulated catecholamine release by inhibiting local renin–angiotensin system modulation. These two drugs also have an additive effect as the reduction in the catecholamine release following their administration decreased from 29–36% to 52%. This additive effect may be of benefit when treating conditions such as hypertension or heart failure. Both candesartan and ramipril had similar outcomes on stimulated catecholamine release. However, angiotensin converting enzyme inhibitors, such as ramipril, because they share a common pathway are known to cause bradykinin accumulation, which has the potential to cause cough (Koji et al., 2003). Thus, candesartan may be advantageous over ramipril because it does not cause cough.

Previously, Critchley et al. (1980) had reported that the stimulated dog adrenal gland secreted noradrenaline to adrenaline in a ratio of 1:4 and quoted a percentage for noradrenaline release of 19%. In comparison, these same authors also showed that the cat adrenal gland selectively released noradrenaline in response to different stimuli such as splanchnic nerve, baroreceptor and chemoreceptor stimulation that ranged from 16% to 74%. The terms selective and non-selective to describe the release were used. Other authors have reported similar percentages of 15% to 20% for electrical stimuli of 1 and 3 Hz using the dog adrenal gland (Foucart et al., 1987; Hosokawa et al., 2000; Koji et al., 2003). In the present study, we reported noradrenaline percentages of 14% to 25% during electrical stimulation, with lower noradrenaline percentages of 7% to 10% for basal release.

The systemic administration of angiotensin II was associated with percentages of noradrenaline release that increased from 30% to 50% as the dose of candesartan increased. However, the dog adrenal gland releases noradrenaline non-selectively and candesartan was not seen to increase the noradrenaline release when the gland was directly stimulated (Table 2). Thus, the higher than expected noradrenaline levels were probably of circulatory in origin, as discussed previously. Presumably, the combination of angiotensin II and candesartan facilitated the release of noradrenaline from the sympathetic nerve endings, which contaminated our adrenal vein samples. Further studies are needed to investigate the true nature of these findings.

In conclusion, both candesartan and ramipril inhibited electrically stimulated catecholamine release and the most likely explanation was blockade of local modulation by the renin–angiotensin system. This effect may prove useful when treating certain medical disorders such as hypertension and heart failure. When using these two drugs in combination as their effects were enhanced, which may also be of clinical benefit. The release of catecholamines from the dog adrenaline gland was non-selective. However, some of our findings were difficult to interpret because drugs were administered systemically and circulating levels of catecholamines were not measured. These two factors need to be taken into account in the design of future studies.

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