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Renal effects of infusion of rilmenidine and guanabenz in conscious dogs: contribution of peripheral and central nervous system α_2-adrenoceptors

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1 We tested the renal effects of the α_2-adrenoceptor agonists, rilmenidine and guanabenz and the antagonists, 2-methoxyidazoxan and idazoxan, in conscious dogs. Our aim was to test the hypothesis that putative imidazoline (I) receptors influence renal function. We reasoned that since rilmenidine and guanabenz are selective for I_1- and I_2-binding sites respectively, an influence of one of these receptive sites on renal function would be reflected in qualitative differences between the effects of these agents. Moreover, effects mediated by putative I-receptors should be relatively resistant to antagonism by the selective α_2-adrenoceptor antagonist, 2-methoxyidazoxan. Since the effects of these drugs on renal function could be mediated in the central nervous system or periphery, the dogs were studied under both normal and ganglion-blocked conditions.

2 In dogs with intact autonomic reflexes, 2-methoxyidazoxan (15 $\mu g\ kg^{-1}$ plus 0.6 $\mu g\ kg^{-1}\ min^{-1}$) produced effects consistent with a generalized increase in sympathetic drive, including increases in mean arterial pressure and plasma renin activity, and a reduction in sodium excretion. In ganglion-blocked dogs, 2-methoxyidazoxan reduced sodium excretion but had no discernible effect on systemic or renal haemodynamics. We conclude that an α_2-adrenoceptor-mediated mechanism in the central nervous system tonically inhibits sympathetic drive in the conscious dog.

3 In ganglion-blocked dogs idazoxan (3–300 $\mu g\ kg^{-1}$) dose-dependently increased arterial pressure. This was not abolished by concomitant administration of 2-methoxyidazoxan (0.3–30 $\mu g\ kg^{-1}$). The pressor effect of idazoxan is therefore probably mediated by an agonist action at α_1-adrenoceptors.

4 The effects of infusions of rilmenidine (0.1–1.0 $mg\ kg^{-1}$) and guanabenz (10–100 $\mu g\ kg^{-1}$) were indistinguishable. They comprised dose-dependent increases in mean arterial pressure, urine excretion, and glomerular filtration rate (the latter in ganglion blocked dogs only), and dose-dependent reductions in heart rate, renal blood flow and sodium excretion (only in dogs with intact autonomic reflexes). All of these effects were antagonized by 2-methoxyidazoxan.

5 We conclude that the renal effects of rilmenidine and guanabenz infusions in conscious dogs are predominantly, if not completely, attributable to activation of α_2-adrenoceptors. Our results do not support the hypothesis that putative I-receptors contribute towards the renal effects of these agents.

Keywords: α_2-Adrenoceptors; central nervous system; conscious dog; imidazoline receptor; kidney; renal blood flow; sodium excretion

Introduction

It is now well established that certain α_2-adrenoceptor ligands bind to non-adrenoceptor (catecholamine insensitive) binding sites which have been claimed to represent a novel pharmacological receptor type, collectively called imidazoline-preferring receptors (Reis *et al.*, 1992). Radioligand binding studies have demonstrated that these binding sites can be broadly classified into two main subtypes. Those which can be labelled with [^{3}H]-clonidine or [^{3}H]-*p*-aminoclonidine, and which have high affinity for imidazoline and oxazoline derivatives such as clonidine, rilmenidine and moxonidine have been termed I_1-binding sites. Those that can be labelled with [^{3}H]-idazoxan, and which have a high affinity for guanidinium derivatives, such as guanabenz, have been termed I_2-binding sites (Reis *et al.*, 1992).

There is some evidence that these binding sites can, at least under certain experimental circumstances, mediate some of the effects of these α_2-adrenoceptor ligands. In particular, there is strong evidence that I_1-receptors in the brainstem can mediate the centrally mediated antihypertensive activity of drugs such as clonidine, rilmenidine and moxonidine (Gomez *et al.*, 1991; Ernsberger *et al.*, 1992; Haxhiu *et al.*, 1994; Sannajust & Head, 1994). There is also evidence that a reduction in renal sympathetic tone induced by these mechanisms could contribute, in the longer term, to the antihypertensive action of drugs such as rilmenidine by enhancing sodium excretion (Kline & Cechetto, 1993; Penner & Smyth, 1994).

I-binding sites have been found in the kidneys of all of the species in which it has been tested. Both I_1- and I_2-subtypes are present in rat and rabbit kidney (Ernsberger *et al.*, 1990; Hamilton *et al.*, 1991; MacKinnon *et al.*, 1993). I_2-subtypes have been observed in the kidneys of human subjects (Lachaud *et al.*, 1992), pigs (Vigne *et al.*, 1989), guinea-pigs (Wikberg *et al.*, 1992) and dogs (Evans & Haynes, 1994). There is also evidence from *in vitro* studies that drugs such as idazoxan and cirazoline, which have high affinity for I_2-binding sites (Coupry *et al.*, 1989), can inhibit tubular Na^+/H^+ exchange systems through a non-adrenoceptor mechanism (Frelin *et al.*, 1986; Bidet *et al.*, 1990). Activation of tubular α_2-adrenoceptors appears to have the opposite effect (Nord *et al.*, 1987). There is also evidence from *in vivo* studies in rats that activation of putative I_1-receptors in the kidney might promote sodium excretion (Smyth *et al.*, 1992; Allan *et al.*, 1993; Li & Smyth, 1993).

In the present study we tested the effects of two α_2-adrenoceptor agonists, which also display high affinity for I_1-(rilmenidine) and I_2- (guanabenz) binding sites respectively (Ernsberger *et al.*, 1992; Evans & Haynes, 1994), on renal function in conscious dogs. Dogs were used, since in this large species it is possible to monitor simultaneously haemody-

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namic, renal and hormonal factors, which might all play a role in the renal effects of these compounds. A chronically prepared conscious preparation was used since this enabled us to study the effects of these agents in the absence of the confounding influence of anaesthesia, and allowed the use of within-animal experimental designs. Drugs were administered as intravenous infusions to optimize drug delivery to both the central nervous system and kidney biophases.

We reasoned that if activation of putative I_1- or I_2-receptors influenced renal function, the renal effects of rilmenidine and guanabenz might differ since rilmenidine has selective affinity for I_1-binding sites and guanabenz has selective affinity for I_2-binding sites. Moreover, it would also be expected that any effects of these agents mediated by putative I-receptors would be relatively resistant to antagonism of α_2-adrenoceptors. Therefore, in order to test the involvement of α_2-adrenoceptors in the actions of these compounds, we examined their effects both in the absence and presence of the highly selective α_2-adrenoceptor antagonist, 2-methoxyidazoxan. Since both central nervous system and renal putative I-receptors could conceivably influence renal function, it was necessary for us to test the involvement of the renal sympathetic innervation in the actions of rilmenidine and guanabenz. We therefore tested their effects under conditions where autonomic reflexes were intact, and following ganglionic blockade.

Methods

All experimental procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990), and were approved in advance by the Alfred Hospital/Baker Medical Research Institute Animal Experimentation Committee. Six male greyhound dogs were used, which weighed between 28.6 and 32.5 kg (mean 30.0) at the time of their preliminary surgery. Each dog underwent a thorough veterinary inspection and was free of parasites and other pathologies before surgery.

Surgical preparation

The preparative surgery was performed under halothane anaesthesia (1–2%, Fluothane, ICI, Melbourne, Australia) after induction with intravenous propofol (6 mg kg^{-1}, Diprivan, ICI, Melbourne, Australia) and endotracheal intubation. Via a left retroperitoneal incision the dogs were equipped with a flow probe (type 6SB, Transonic Systems Inc., Ithaca, New York, U.S.A.) around the left renal artery, two catheters in the abdominal aorta (Herd & Barger, 1964), and two catheters in the inferior vena cava. The right kidney was removed via a right retroperitoneal incision. Post-operative medications comprised morphine (1.25–5 mg, s.c. every 8 h for up to 48 h. David Bull Laboratories, Victoria, Australia), flunixin (50 mg every 12 h for up to 24 h; Finadyne, Heriot Agvet, Victoria, Australia) and amoxycillin (500 mg orally three times daily for 7–10 days; Alphamox, Alphapharm, Queensland, Australia).

Prior to the surgery, the dogs were accustomed to the laboratory environment and were trained to lie on a padded table. Post-operatively, the catheters were flushed daily (for 10 days) with the dogs lying on the experimental table, to ensure that the animals were relaxed in the laboratory environment.

Measurement of haemodynamic variables

Arterial pressure and central venous pressure were measured by connecting the aortic and vena caval catheters respectively to a disposable transducer (Cobe, Arvarda, U.S.A.) zeroed at the level of the dogs heart. Heart rate was measured by a tachometer activated by the arterial pressure pulse. Renal blood flow was measured by connecting the implanted flow probe to a flow meter (model T108, Transonic Systems Inc., New York, U.S.A.). Signals were amplified and recorded on a Neotrace pen recorder (Neomedix Systems, Sydney, Australia), and then relayed to an Olivetti M28 computer with an analogue-to-digital converter which provided 20 s means of each variable.

Experimental protocols

General Each dog underwent 6 to 18 studies, performed at 4 to 7 day intervals. The order of each of the studies was randomized. Each study followed the same basic protocol. Between 08 h 00 min and 08 h 30 min, with the dog lying on the experimental table, a catheter (6 French gauge, Arnolds Veterinary Products Pty Ltd, Reading, England) was lubricated with local anaesthetic jelly (2% lignocaine, Astra Pharmaceuticals, NSW, Australia) and inserted into the bladder via the urethra. Samples of urine (5 ml) and blood (5 ml) were then taken to determine background levels of [^{3}H]-inulin and para-aminohippuric acid (PAH). Each dog was then administered (i.v.) bolus doses of [^{3}H]-inulin (30 μCi), PAH (75 mg), and in some studies pentolinium (6 mg kg^{-1}) for autonomic ganglion blockade. An intravenous infusion of 5% w/v dextrose at a rate of 1 ml min^{-1} was then begun and continued for the duration of the study. This solution contained [^{3}H]-inulin (0.4 μCi ml^{-1}), PAH (3.3 mg ml^{-1}), and pentolinium (1.5 mg ml^{-1}; only in studies requiring ganglion blockade). The dosage regimen of pentolinium we used was shown previously to prevent completely the heart rate response to a dose of glyceryl trinitrate which caused profound hypotension (Anderson *et al.*, 1985).

Seventy five minutes later the first of four 30 min experimental periods was begun. Drugs (guanabenz, rilmenidine, 2-methoxyidazoxan or idazoxan) were administered as intravenous infusions during each of these periods. During the first 5 min of the experimental period a loading dose was given as an infusion at 25 μl kg^{-1} min^{-1}. For the remainder of the experimental period the rate of drug infusion was 5 μl kg^{-1} min^{-1}. This protocol was followed so that haemodynamic variables would be stable over the final 20 min of each experimental period, during which clearance measurements were made (clearance period). The urine produced during these 20 min clearance periods was collected, and blood samples (20 ml) were taken half-way through each clearance period for renal function and hormonal measurements (see *Analysis of urine and blood samples*). Plasma volume was replaced whenever blood was collected, by intravenous administration of an equivalent volume of a polygeline/electrolyte solution (Haemaccel, Hoechst Australia Ltd, Victoria).

Protocol 1 Dose-dependent effects of 2-methoxyidazoxan and idazoxan in ganglion-blocked dogs This protocol involved four studies performed in random order. The aim was to test for pressor (α-agonist) effects of these drugs which could confound the interpretation of subsequent experiments. The dogs received no treatment during experimental period 1 and then cumulative doses were administered over the 2nd, 3rd and 4th experimental periods of either vehicle (5% w/v dextrose), or 2-methoxyidazoxan (0.3, 3.0 and 30 μg kg^{-1}), idazoxan (3, 30, and 300 μg kg^{-1}), or both agents given simultaneously. These doses were administered in a volume of 125 μl kg^{-1} over 5 min followed by a constant infusion of 5 μl kg^{-1} min^{-1} for the rest of the experimental period.

Protocol 2 Dose-dependent effects of guanabenz and rilmenidine, with or without 2-methoxyidazoxan pretreatment, in dogs with intact autonomic nervous systems This protocol involved 6 studies, performed in random order. The aim was to examine the effects of rilmenidine and guanabenz on renal function, and to test whether any of these effects are resistant to antagonism by the selective α_2-adrenoceptor antagonist, 2-methoxyidazoxan. At the beginning of the first experimental period, the dogs were treated with either 2-methoxyidazoxan (15 μg kg^{-1} administered over 5 min plus 0.6 μg kg^{-1} min^{-1} for

the rest of the study) or its vehicle (5% w/v dextrose; 125 μl kg^{-1} administered over 5 min plus 5 μl kg^{-1} min^{-1} for the rest of the study). At the beginning of each of the subsequent experimental periods the dogs were treated with either guanabenz (total cumulative doses of 10, 30 and 100 μg kg^{-1}), rilmenidine (total cumulative doses of 0.1, 0.3 and 1 mg kg^{-1}), or vehicle (5% w/v dextrose; 25 μl kg^{-1} min^{-1} for 5 min plus 5 μl kg^{-1} min^{-1} for the remaining 25 min of each period).

Protocol 3 Dose-dependent effects of guanabenz and rilmenidine, with or without 2-methoxyidazoxan pretreatment, in ganglion blocked dogs. This protocol was identical to protocol 2, but the dogs were ganglion-blocked by administration of pentolinium (see above). The aim of this study was to examine the autonomic nervous system-independent effects of rilmenidine and guanabenz, and to test whether any of these effects are resistant to antagonism by 2-methoxyidazoxan.

Protocol 4 Dose-dependent effects of 2-methoxyidazoxan during administration of guanabenz, rilmenidine or vehicle in ganglion-blocked dogs This protocol involved 4 studies, performed in random order. The aim of this protocol was similar to that of protocol 3 (above), except that lower doses, and more prolonged infusions, of rilmenidine and guanabenz were tested. Moreover, the order in which the agonists and 2-methoxyidazoxan were administered was reversed. It was necessary to test relatively low doses of guanabenz and rilmenidine since ganglion blockade greatly augmented the pressor effects of these agents, which could confound the interpretation of these experiments. We tested the effects of more prolonged (90 min) infusions of these relatively low doses since some of the renal effects of these agents could conceivably take some time to be expressed. The dogs received no treatment during experimental period 1, but at the start of experimental period 2, an infusion of either guanabenz (5 μg kg^{-1} over 5 min followed by a constant infusion of 0.2 μg kg^{-1} min^{-1} for the rest of the study) or rilmenidine (50 μg kg^{-1} over 5 min followed by a constant infusion of 2 μg kg^{-1} min^{-1} for the rest of the study) commenced. Cumulative doses of either 2-methoxyidazoxan (0.3, 3.0 and 30 μg kg^{-1}) or its vehicle (5% w/v dextrose) were administered over the second, third and fourth experimental periods. These were administered in a volume of 25 μl kg^{-1} min^{-1} for 5 min followed by a constant infusion of 5 μl kg^{-1} min^{-1} for the rest of the experimental period.

Analysis of urine and blood samples

From each 20 ml blood sample, a small quantity was used for measurement of haematocrit by the capillary tube method. The remainder was used for measurement of plasma levels of renin activity (3 ml; Oliver *et al.*, 1990), and atrial natriuretic peptide (5 ml; Woods, 1988), [^{3}H]-inulin, PAH, sodium and potassium concentration (see below).

Urine volume was measured gravimetrically. PAH concentrations in urine and plasma were measured by the method of Smith *et al.* (1945). Sodium and potassium concentrations were measured by flame photometry (Instrument Laboratory 943, Italy). [^{3}H]-inulin concentrations were quantified by liquid scintillation counting (see Evans *et al.*, 1994).

Calculation of derived renal function variables

Filtration fraction, and the fractional excretion of sodium and potassium were calculated as described previously (Woods & Anderson, 1990), and expressed as a percentage. The fractional excretion of urine was defined as (100 × urine flow)/(glomerular filtration rate).

Calibration of flow probes in vivo

In studies of renal function it is imperative to measure renal blood flow both accurately and precisely. Because we measured renal blood flow both by ultrasonic doppler flowmetry and indirectly by the renal clearance of PAH, it was possible for us to compare the values obtained by the two methods. We reasoned that although measurement of effective renal plasma flow by PAH clearance is relatively imprecise owing to difficulties in completely evacuating the bladder of conscious dogs and also possibly to variability in PAH extraction across the kidney, when averaged over a series of clearance periods it should enable accurate estimates of renal blood flow. On the other hand, although the renal blood flow measurements obtained by doppler flowmetry are likely to be precise, their accuracy depends on their calibration. The calibration of each of the probes used in the present study was tested *in vitro* by a method used previously (Evans *et al.*, 1992). We found that *in vitro* each probe had a negligible zero offset and gave readings between 102 and 121% of the actual flow, which is within the manufacturers specifications. *In vivo*, however, there were large differences between the flow values given by doppler flowmetry and PAH clearance. Moreover, although the ratios of the two sets of measurements were different in each dog, they were relatively constant within each dog across study days. Thus, in each of the six dogs studied, the average values of renal blood flow determined by doppler flowmetry were 62, 35, 24, and 11% less, and 48 and 58% more, respectively, than those obtained by PAH clearance. This was not due to baseline error, since zero offset was < 8 ml min^{-1} immediately after death in each dog. Thus, we concluded that the discrepancy was due to errors in the calibration (gain) of the probes. Therefore we corrected all doppler flowmetry measurements of renal blood flow by multiplying them by the ratio of the average (across the days experiment) levels of renal blood flow estimated by the PAH clearance technique and the doppler flowmetry technique. We contend that this technique provides both accurate and precise estimates of renal blood flow.

Statistical analysis

The statistical computer software package SYSTAT (Wilkinson, 1990) was used for statistical analyses. The levels of the variables during the first experimental period were compared across treatment groups within each protocol by analysis of variance. The dose-dependent effects of guanabenz, rilmenidine, 2-methoxyidazoxan and idazoxan were tested for by repeated measures analysis of variance (see Ludbrook, 1994). The interaction term, between the drug administered and its dose, was used as the test statistic, and P values were conservatively adjusted by the Greenhouse-Geisser correction (see Ludbrook, 1994). When multiple comparisons were made within a single experiment, the Dunn-Sidak correction was applied to protect against the increased risk of type 1 error. Because the effects of guanabenz and rilmenidine were indistinguishable (see Results), P values testing for the effects of these drugs were combined by the method of Sokal & Rohlf (1981), and when they did not derive from independent tests, conservatively adjusted using the Dunn-Sidak correction. All variables are expressed as the between-dog mean ± s.e.mean.

Drugs and analytical chemicals

The following drugs were used during this study: pentolinium tartrate (Institute of Drug Technology, Victoria, Australia), rilmenidine dihydrogen orthophosphate (a gift from Servier, France), idazoxan HCl (a gift from Reckitt & Colman Ltd, Hull, East Yorkshire, UK), guanabenz acetate and 2-methoxyidazoxan (Research Biochemicals Inc., U.S.A.).

[^{3}H]-inulin (New England Nuclear, Sydney, Australia) was purified by dialysis as described previously (Evans *et al.*, 1994). PAH was obtained from the Sigma Chemical Company (St Louis MO, U.S.A.).

Results

Resting haemodynamic, renal and hormonal variables, and the effect of ganglion blockade

The resting levels of these variables in the 4 conscious dogs studied both with and without ganglion blockade are shown in Table 1. These were within the normal range for our laboratory (Woods, 1988; Woods & Anderson, 1990). In response to ganglion blockade heart rate increased on average by 55±6 beats min^{-1}, glomerular filtration rate was reduced by 14±4 ml min^{-1}, fractional urine excretion increased by 1.15±0.20%, the fractional excretion of sodium increased by 2.37±0.60%, and potassium excretion and the fractional excretion of potassium increased by 23±4 μmol min^{-1} and 30.5±4.2% respectively. There were also tendencies for filtration fraction to fall (by 13±5% ($P=0.07$)) and sodium excretion to rise (by 63±31 μmol min^{-1} ($P=0.1$)). Ganglion blockade had no effect on mean arterial pressure, central venous pressure, renal blood flow, haematocrit or urine flow.

Effects of 2-methoxyidazoxan and idazoxan

In Protocol 1 we tested the effects of a range of doses of 2-methoxyidazoxan and idazoxan in ganglion-blocked dogs, in order to test whether these agents have any pressor (α-agonist) effects that could confound the interpretation of subsequent experiments. We used 10 fold higher doses of idazoxan than of 2-methoxyidazoxan since the latter has 10 fold higher affinity for α_2-adrenoceptors in dog kidney (Evans & Haynes, 1994). The effects of 2-methoxyidazoxan (0.3–30 μg kg^{-1}) were indistinguishable from those of vehicle, in that mean arterial

Table 1 Baseline levels of haemodynamic and renal function variables in conscious dogs: effects of ganglion blockade

Treatment	*Intact autonomic nervous system*	*Ganglionic blockade*
Body wt (kg)	28.5±0.6	28.7±0.6
MAP (mmHg)	98±3	100±4
HR (beats min^{-1})	69±5	123±5**
CVP (mmHg)	0.5±0.4	1.0±0.3
Hct (%)	41±2	39±1
RBF (ml min^{-1})	208±5	193±17
GFR (ml min^{-1})	41±4	27±1*
Filtration fraction (%)	37±6	24±2
Urine flow (ml min^{-1})	0.45±0.10	0.61±0.03
$U_{Na}V$ (μmol min^{-1})	97±10	156±21
U_KV (μmol min^{-1})	26±3	50±3*
%urine excretion (%)	1.11±0.21	2.26±0.06**
%Na excretion (%)	1.65±0.23	4.02±0.42*
%K excretion (%)	16.5±1.9	47.0±3.0**

Each value is the between-dog mean±s.e.mean of average values during the first clearance period in three separate experiments for 4 dogs. Ganglion blockade was induced by administration of pentolinium (6 mg kg^{-1} bolus plus 3 mg kg^{-1} h^{-1}). Body wt., body weight; MAP, mean arterial pressure; HR, heart rate; CVP, central venous pressure; Hct, haematocrit; RBF, renal blood flow; GFR, glomerular filtration rate; $U_{Na}V$, sodium excretion rate; U_KV, potassium excretion rate; %Na, fractional excretion of sodium; %K, fractional excretion of potassium. For definition of clearance measurements see Methods. *$P\leq0.05$, **$P\leq0.01$ for comparison between levels in the absence and presence of ganglion blockade (d.f. 1,3).

Table 2 Baseline levels of haemodynamic and renal function variables in conscious dogs: effect of 2-methoxyidazoxan (2-MI)

	Intact autonomic nervous system		*Ganglionic blockade*	
Treatment	*Vehicle*	*2-MI*	*Vehicle*	*2-MI*
Body wt. (kg)	29.3±0.9	29.3±0.9	29.6±1.1	29.6±1.1
MAP (mmHg)	101±5	119±8*	98±3	99±5
HR (beats min^{-1})	68±5	87±9	115±8	114±8
CVP (mmHg)	1.1±0.7	0.7±0.6	0.1±0.9	-0.5±0.9
Hct (%)	42±1	48±2*	40±1	42±1
RBF (ml min^{-1})	207±12	249±16	179±19	191±31
GFR (ml min^{-1})	41±3	39±6	29±2	27±3
Filtration fraction (%)	37±5	30±4	31±7	27±3
Urine flow (ml min^{-1})	0.45±0.07	0.61±0.23	0.62±0.02	0.49±0.07
$U_{Na}V$ (μmol min^{-1})	96±8	42±7**	143±23	110±16*
U_KV (μmol min^{-1})	28±3	22±8	49±3	55±13
%urine excretion (%)	1.09±0.16	1.35±0.25	2.26±0.04	2.01±0.06
%Na excretion (%)	1.62±0.18	0.86±0.1*	4.23±0.39	3.05±0.14*
%K excretion (%)	17.6±1.8	14.1±2.5	47.8±2.5	56.4±4.6
PRA (ng AI ml^{-1} h^{-1})	0.21±0.04	0.59±0.01***		
ANP (pg ml^{-1})	23.3±5.9	16.8±2.2		

Each value is the between-dog mean±s.e.mean of average values for 5 dogs during the first clearance period in three separate experiments for 5 dogs. 2-MI was administered as an infusion of 3 μg kg^{-1} min^{-1} for 5 min (15 μg kg^{-1}) followed by an infusion of 0.6 μg kg^{-1} min^{-1} for the rest of the experiment. Ganglion blockade was induced by administration of pentolinium (6 mg kg^{-1} bolus plus 3 mg kg^{-1} h^{-1}). Abbreviations as for Table 1, except for plasma renin activity (PRA) and atrial natriuretic peptide (ANP). AI = angiotensin I. *$P\leq0.05$, **$P\leq0.01$, ***$P\leq0.001$ for comparison between levels in the absence and presence of 2-methoxyidazoxan (d.f. 1,4). Note that hormone measurements (PRA and ANP) were not made in ganglion-blocked dogs.

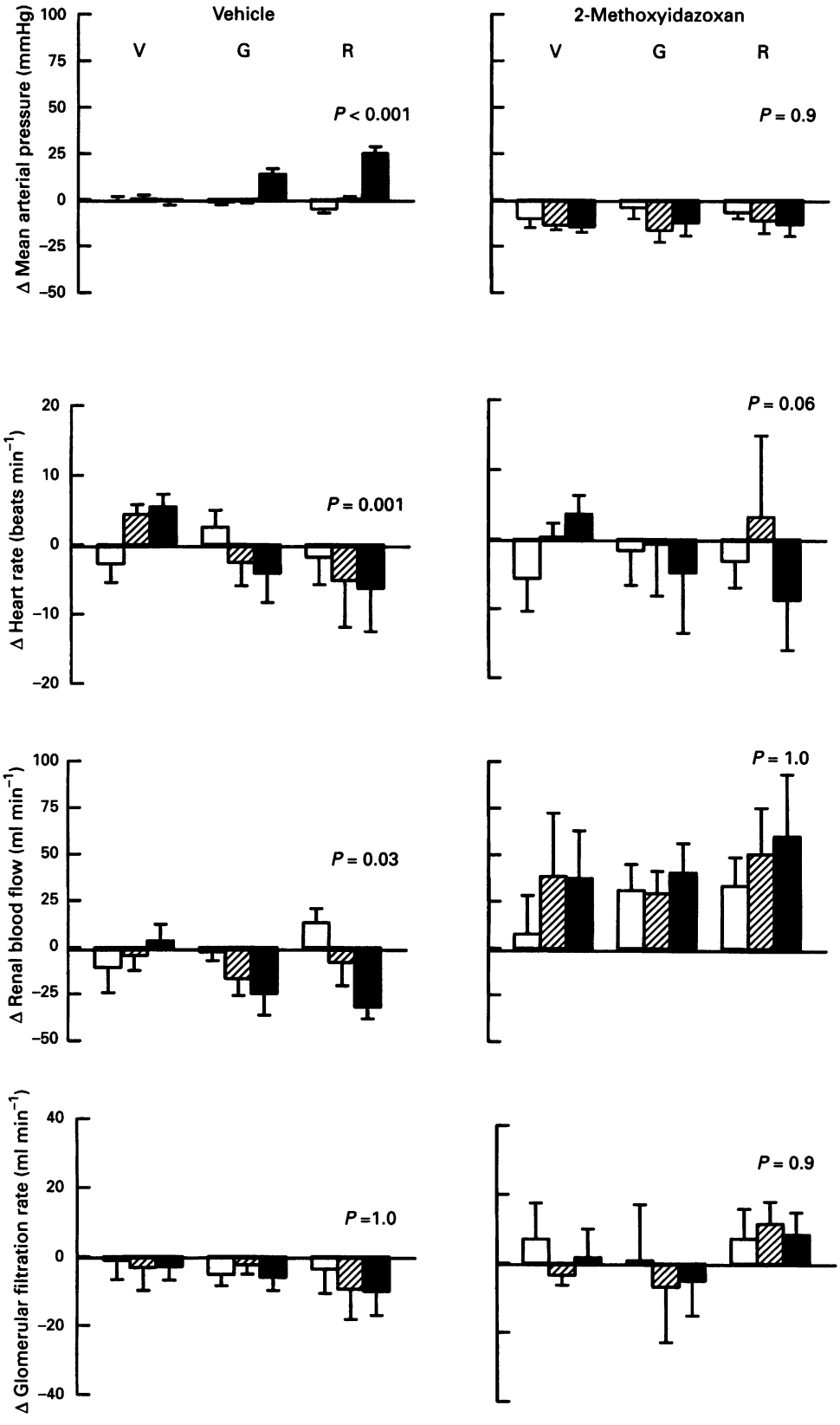

Figure 1 Changes in the levels of mean arterial pressure, heart rate, renal blood flow and glomerular filtration rate in dogs with intact ganglionic neurotransmission, in response to vehicle (V), or ascending doses of guanabenz (G) and rilmenidine (R). The treatments and their order were: (open column), vehicle, 10 μg kg^{-1} guanabenz, 100 μg kg^{-1} rilmenidine; (hatched column), vehicle, 30 μg kg^{-1} guanabenz, 300 μg kg^{-1} rilmenidine; (solid column), vehicle, 100 μg kg^{-1} guanabenz, 1 mg kg^{-1} rilmenidine. The panels on the right hand side show responses following 2-methoxyidazoxan pretreatment (bolus of 15 μg kg^{-1} administered over 5 min plus a constant infusion of 0.6 μg kg^{-1} min^{-1} for the rest of the study), while the panels on the left show responses after vehicle treatment. *P* values represent the outcomes of repeated measures analysis of variance (d.f 2,16) for non-parallelism between vehicle-treatment and guanabenz- and rilmenidine-treatment. Because the effects of guanabenz and rilmenidine were qualitatively similar, the *P* values for their effects were combined by the method of Sokal & Rohlf (1981), and the resultant *P* values were conservatively adjusted with the Dunn-Sidak correction (see Ludbrook, 1994).

pressure, heart rate, renal blood flow, glomerular filtration rate, filtration fraction, sodium excretion, and the fractional excretion of urine and sodium did not change. In contrast idazoxan, at cumulative doses of 3, 30 and 300 μg kg^{-1}, increased mean arterial pressure by 5±2, 12±3 and 49±3 mmHg respectively. This dose-dependent pressor effect

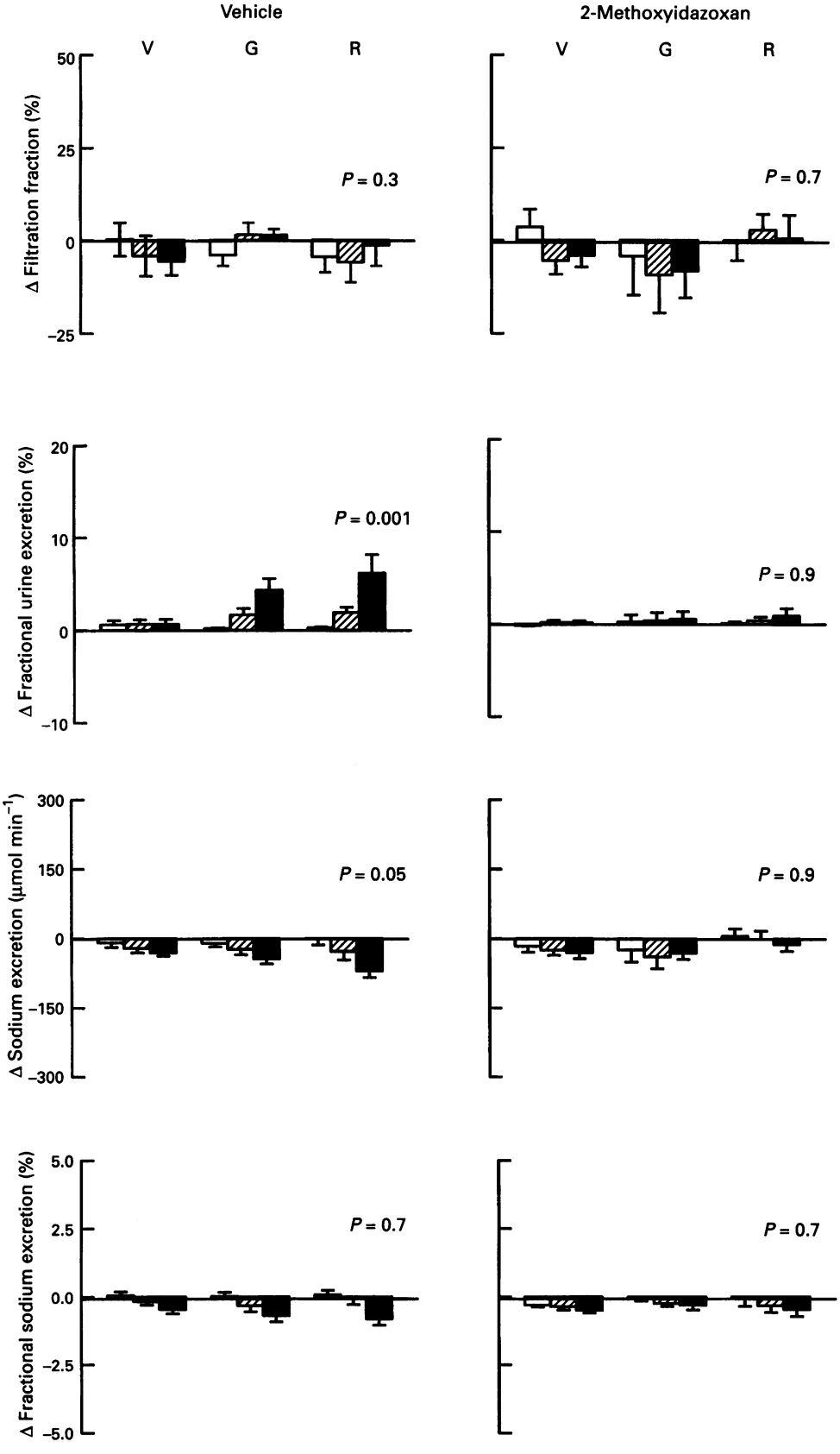

Figure 2 Changes in the levels of filtration fraction, fractional urine excretion, sodium excretion and fractional sodium excretion in dogs with intact ganglionic neurotransmission, in response to vehicle, or ascending doses of guanabenz and rilmenidine. The treatments and *P* values are the same as those in Figure 1.

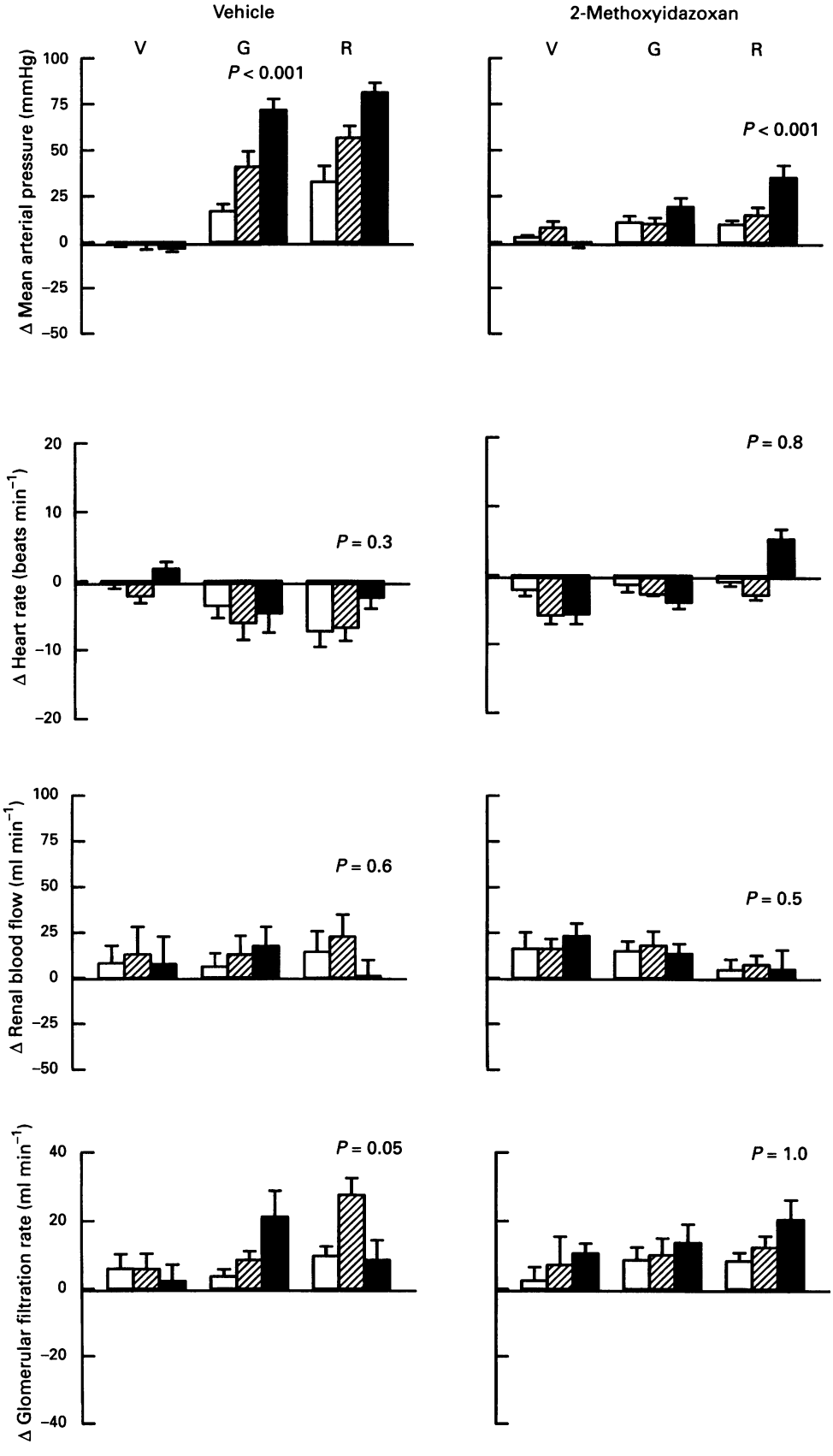

Figure 3 Changes in the levels of mean arterial pressure, heart rate, renal blood flow and glomerular filtration rate in ganglion-blocked dogs, in response to vehicle, or ascending doses of guanabenz and rilmenidine. Neurotransmission in autonomic ganglia was blocked by administration of pentolinium (6 mg kg^{-1} plus 1.5 mg min^{-1}). The treatments and *P* values are the same as those in Figure 1.

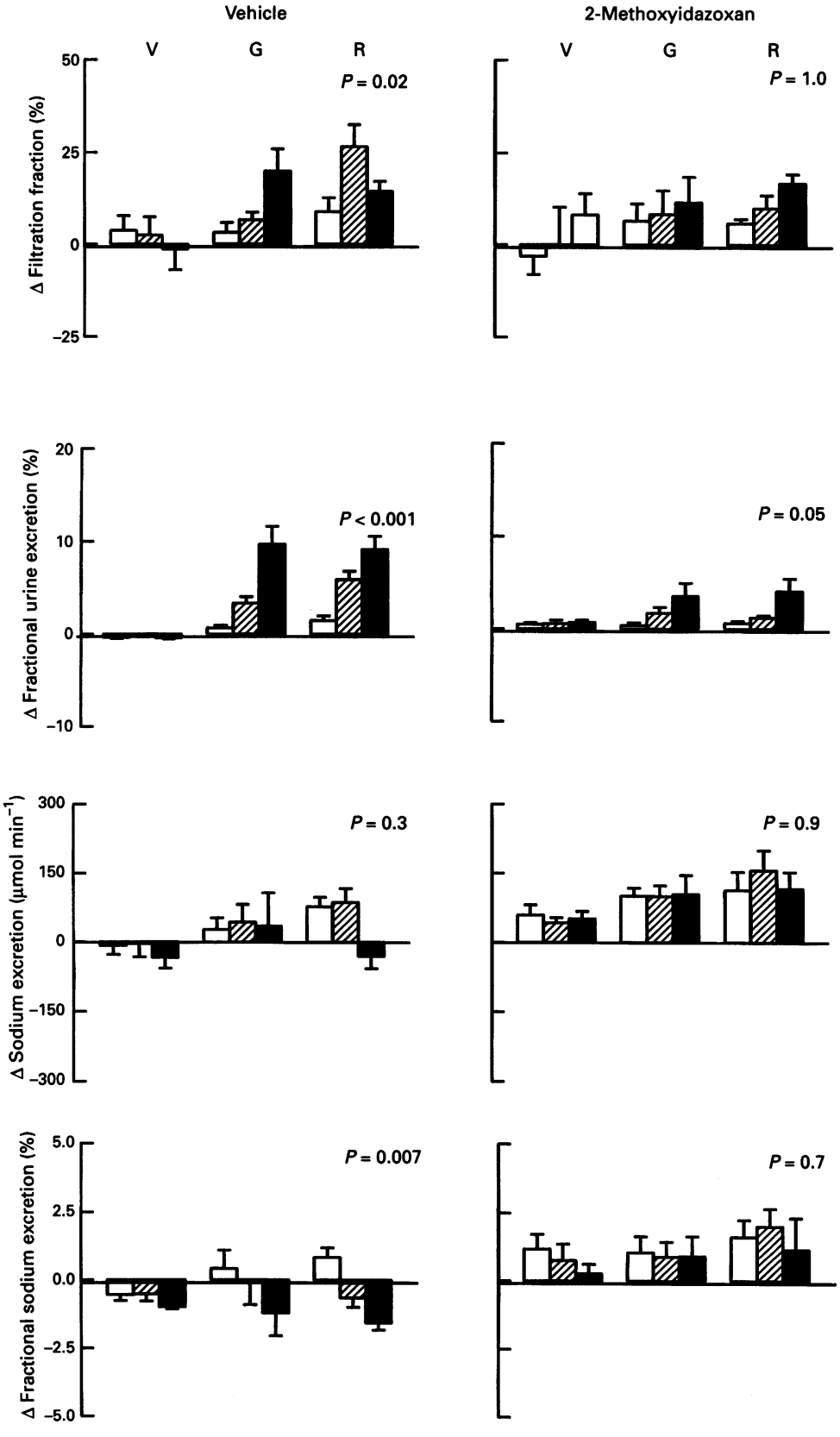

Figure 4 Changes in the levels of filtration fraction, fractional urine excretion, sodium excretion and fractional sodium excretion in ganglion-blocked dogs, in response to vehicle, or ascending doses of guanabenz and rilmenidine. Neurotransmission in autonomic ganglia was blocked by administration of pentolinium ($6\,mg\,kg^{-1}$ plus $1.5\,mg\,min^{-1}$). The treatments and P values are the same as those in Figure 1.

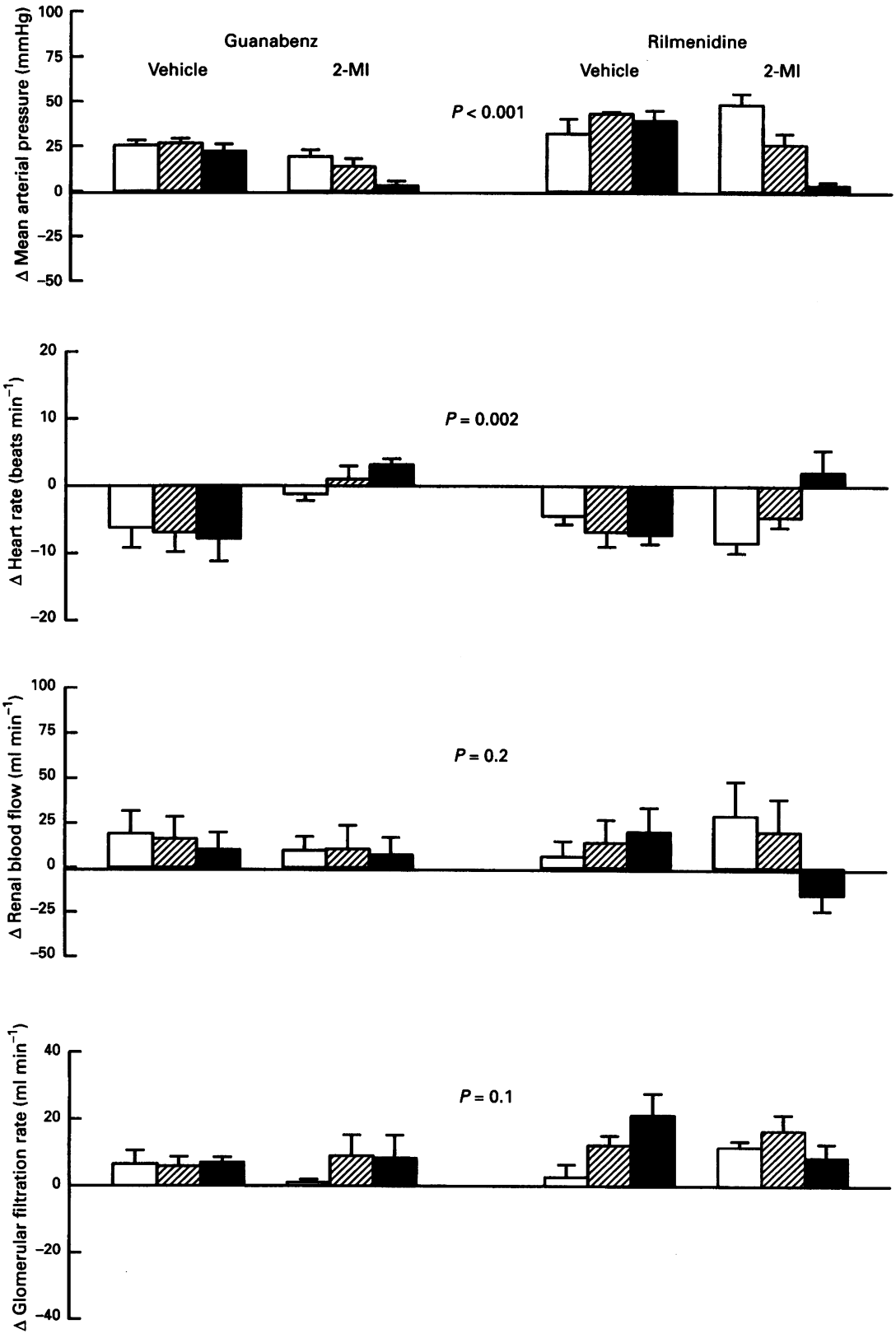

Figure 5 Changes in the levels of mean arterial pressure, heart rate, renal blood flow, and glomerular filtration rate in ganglion-blocked dogs in response to ascending doses of 2-methoxyidazoxan (2-MI) or its vehicle with concomitant infusion of guanabenz or rilmenidine. The treatments and their order were: (open column), vehicle or 0.3 $\mu g\,kg^{-1}$ 2-MI; (hatched column), vehicle or 3 $\mu g\,kg^{-1}$ 2-MI; (solid column), vehicle or 30 $\mu g\,kg^{-1}$ 2-MI. The responses to 2-MI were tested following pretreatment with either guanabenz (5 $\mu g\,kg^{-1}$ bolus over 5 min plus a constant infusion of 0.2 $\mu g\,kg^{-1}\,min^{-1}$ for the rest of the study), or rilmenidine (50 $\mu g\,kg^{-1}$ bolus over 5 min plus a constant infusion of 2 $\mu g\,kg^{-1}\,min^{-1}$ for the rest of the study). *P* values represent the outcomes of repeated measures analysis of variance (d.f. 2,16) testing for non-parallelism between 2-MI-treatment and vehicle-treatment. Because the actions of guanabenz and rilmenidine were qualitatively similar, the *P* values for the effects of 2-MI on their actions were combined by the method of Sokal & Rohlf (1981).

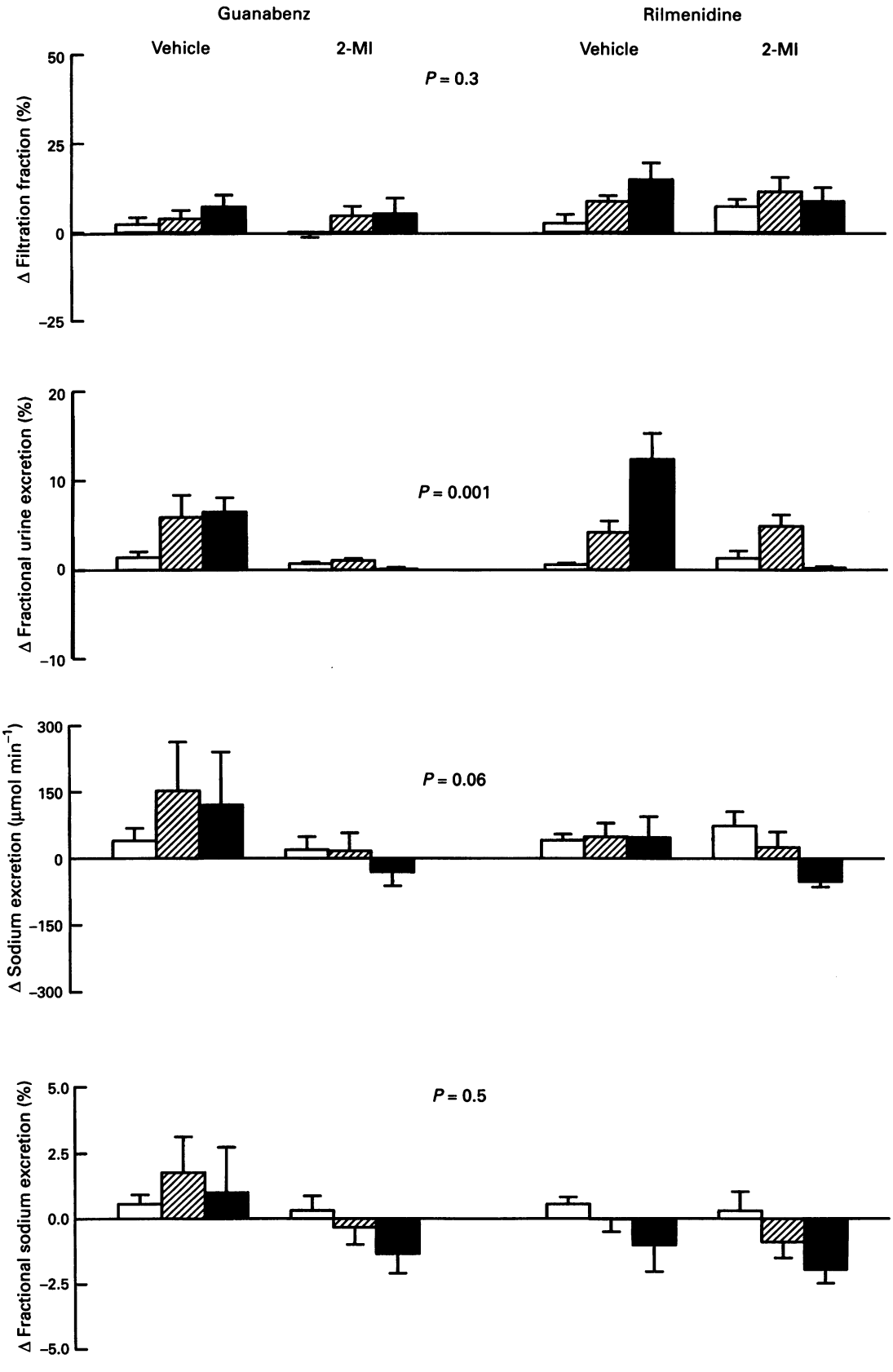

Figure 6 Changes in the levels of filtration fraction, fractional urine excretion, sodium excretion and fractional sodium excretion in ganglion-blocked dogs in response to ascending doses of 2-methoxyidazoxan (2-MI) or its vehicle, with concomitant infusion of guanabenz or rilmenidine. The treatments and *P* values are as for Figure 5.

of idazoxan was still evident when the drug was co-administered with 2-methoxyidazoxan.

In Protocols 2 and 3 we tested the effects of 2-methoxyidazoxan (15 μg kg^{-1} plus 0.6 μg kg^{-1} min^{-1}) in dogs with intact autonomic reflexes and in ganglion blocked dogs (Table 2). In dogs with intact autonomic reflexes, 2-methoxyidazoxan increased mean arterial pressure (by 18±5 mmHg), haematocrit (6±1%), and plasma renin activity (0.38±0.04 ng angiotensin I ml^{-1} h^{-1}), and reduced both sodium excretion (54±7 μmol min^{-1}) and the fractional excretion of sodium (0.76±0.17%). There was also a tendency for heart rate to increase (20±8 beats min^{-1}; $P=0.08$). 2-Methoxyidazoxan had no statistically significant effects on central venous pressure, renal blood flow, glomerular filtration rate, filtration fraction, the absolute or fractional excretions of urine or potassium, or on plasma atrial natriuretic peptide concentration. In ganglion-blocked dogs, the only observed effects of 2-methoxyidazoxan were reductions in sodium excretion (34±12 μmol min^{-1}) and the fractional excretion of sodium (1.18±0.28%) (Table 2).

Dose-dependent effects of guanabenz and rilmenidine

Dogs with intact autonomic reflexes (Protocol 2) The effects of rilmenidine (100, 300 and 1000 μg kg^{-1}) and guanabenz (10, 30 and 100 μg kg^{-1}) were indistinguishable (Figures 1 and 2). Both agents had no effect on mean arterial pressure at the two lower doses, but increased arterial pressure by, respectively, 14±3 mmHg (guanabenz) and 25±3 mmHg (rilmenidine) at the highest doses tested. Guanabenz and rilmenidine also dose-dependently reduced heart rate (by a mean of up to 6 beats min^{-1}), renal blood flow (by up to 32 ml min^{-1}), and sodium excretion (by up to 70 μmol min^{-1}), and increased the fractional excretion of urine (by up to 6.13%). Neither agent affected glomerular filtration rate, filtration fraction, or the fractional excretion of sodium (Figures 1 and 2) or potassium excretion, plasma renin activity, or the plasma concentration of atrial natriuretic peptide (data not shown). Neither rilmenidine nor guanabenz affected any of the measured variables when the dogs were pretreated with the α_2-adrenoceptor antagonist, 2-methoxyidazoxan (Figures 1 and 2).

Ganglion-blocked dogs (Protocol 3)

The effects of guanabenz and rilmenidine were also indistinguishable during ganglion blockade, but were somewhat different from those in dogs with intact autonomic reflexes (Figures 3 and 4). Arterial pressure was dose-dependently increased (by a mean of up to 81 mmHg), as was glomerular filtration rate (28 ml min^{-1}), filtration fraction (27%), and fractional urine excretion (9.71%). Neither agent affected heart rate, renal blood flow, or sodium excretion. Fractional sodium excretion, however, was dose-dependently reduced by a mean of up to 1.57±0.25%. Following pretreatment with 2-methoxyidazoxan, guanabenz and rilmenidine increased arterial pressure and fractional urine excretion, but these increases were less than half those seen in the absence of 2-methoxyidazoxan. No other effects of guanabenz or rilmenidine treatment were observed following 2-methoxyidazoxan pretreatment (Figures 3 and 4).

Dose-dependent effects of 2-methoxyidazoxan during administration of guanabenz or rilmenidine in ganglion-blocked dogs (Protocol 4)

Guanabenz (5 μg kg^{-1} plus 0.2 μg kg^{-1} min^{-1}) and rilmenidine (50 μg kg^{-1} plus 2 μg kg^{-1} min^{-1}) had qualitatively and quantitatively similar effects (Figures 5 and 6). Arterial pressure was increased, and was maintained at mean levels, respectively, 25–22 mmHg (guanabenz) and 32–43 mmHg (rilmenidine) above resting. There was also a small fall in heart rate following both agents, which was maintained at levels, respectively, 6–8 beats min^{-1} (guanabenz) and 4–8 beats min^{-1} (rilmenidine) below resting. Fractional urine excretion rose progressively during the 90 min of infusion, to mean levels 6.52% (guanabenz) and 12.39% (rilmenidine) respectively greater than resting. Neither guanabenz nor rilmenidine affected renal blood flow, glomerular filtration rate, filtration fraction, sodium excretion or the fractional excretion of sodium. The α_2-adrenoceptor antagonist, 2-methoxyidazoxan dose-dependently reversed the hypertensive, bradycardic and polyuric effects of both guanabenz and rilmenidine.

Discussion

We tested the renal effects of intravenous infusion of two centrally acting antihypertensive agents with differing pharmacological profiles. Guanabenz is an α_2-adrenoceptor agonist with relatively low affinity for I_1-binding sites but high affinity for I_2-binding sites (Ernsberger *et al.*, 1992; Evans & Haynes, 1994). In contrast, rilmenidine is an α_2-adrenoceptor agonist with high affinity for I_1-binding sites but relatively low affinity for I_2-binding sites (Ernsberger *et al.*, 1992; Evans & Haynes, 1994). We have also tested whether the effects of these agents are antagonized by the α_2-adrenoceptor antagonist, 2-methoxyidazoxan, which has extremely low affinity for I_2-binding sites (Miralles *et al.*, 1993; Evans & Haynes, 1994). Our aim was to test the hypothesis that these drugs could exert effects on the kidney via activation of putative I_1- (rilmenidine) or I_2- (guanabenz) receptors. Our rationale was that if this hypothesis were true, rilmenidine and guanabenz might have qualitatively different effects on renal function, and that some of these effects would be relatively resistant to antagonism by 2-methoxyidazoxan. We conclude that our experiment provides no support for a role of putative I-receptors in the effects of either rilmenidine or guanabenz on renal function in conscious dogs, since the effects of these agents were indistinguishable and similarly sensitive to antagonism by 2-methoxyidazoxan.

Effects of guanabenz and rilmenidine

Both guanabenz and rilmenidine dose-dependently increased arterial pressure. This effect was more pronounced during ganglionic blockade, indicating that autonomic reflexes, and probably also direct centrally mediated sympathoinhibitory effects (Baum & Shropshire, 1976), normally blunt the peripherally mediated pressor effects of these agents. Pressor, rather than depressor effects were seen, presumably because administration of these drugs by infusion favoured their peripheral vasoconstrictor action over their central sympathoinhibitory action. This notion is supported by the observations of others that both agents lower arterial pressure in dogs under experimental conditions different from those used in the present investigation (Strandhoy *et al.*, 1982; Laubie *et al.*, 1985). The reduction in heart rate seen with these agents in dogs with intact autonomic nervous systems probably represents the combined actions of the reflex response to baroreceptor loading, and a direct action within the brainstem to inhibit cardiac sympathetic drive (see Baum & Shropshire, 1976). However, we cannot exclude the possibility of a direct negative chronotropic effect on the cardiac pacemaker, since we found that in ganglion-blocked dogs these agents had a small (4–8 beats min^{-1}) bradycardiac effect that was dose-dependently antagonized by 2-methoxyidazoxan. In dogs with intact autonomic reflexes both guanabenz and rilmenidine dose-dependently reduced renal blood flow. This most likely represents a direct renal vasoconstrictor action of these agents. In ganglion-blocked dogs no fall in renal blood flow was observed, perhaps because concomitant increases in mean arterial pressure enhanced renal blood flow. Glomerular filtration rate and filtration fraction were dose-dependently increased by guanabenz and rilmenidine in ganglion-blocked dogs. We attribute this to an increase in glomerular capillary pressure secondary to increased arterial pressure, since glomerular filtration did not increase in dogs with intact autonomic reflexes,

in which the pressor effects of guanabenz and rilmenidine were blunted. Guanabenz and rilmenidine also dose-dependently increased fractional urine excretion. Inhibition of water reabsorption, particularly in collecting tubules, via inhibition of vasopressin-induced adenosine 3':5'-cyclic monophosphate formation, is a well known effect mediated through α_2-adrenoceptors (Gellai, 1990). Guanabenz and rilmenidine also reduced sodium excretion in dogs with intact autonomic reflexes. This effect is probably mediated directly within the renal tubule, where activation of α_2-adrenoceptors enhances Na^+ transport (Nord *et al.*, 1987). The lack of an antinatriuretic effect of these agents in ganglion-blocked dogs probably reflects the competing influences of this direct antinatriuretic influence and the natriuretic influence of increased arterial pressure (see Anderson *et al.*, 1995).

Guanabenz was used in the present study as a tool to activate putative I_2-receptors. Guanabenz has high affinity for both α_2-adrenoceptors and I_2-binding sites (Brown *et al.*, 1990; Evans & Haynes, 1994), but is generally reckoned to have low affinity for I_1-binding sites. Thus, it has extremely low affinity for the I_1-binding sites in bovine ventrolateral medullae that have been labelled with [^{3}H]-clonidine and [^{3}H]-*p*-aminoclonidine (Ernsberger *et al.*, 1990; Gomez *et al.*, 1991; Ernsberger *et al.*, 1992; 1993). It also has low affinity for non-adrenoceptor [^{3}H]-clonidine binding sites in bovine adrenal medulla (Molderings *et al.*, 1993). In contrast, guanabenz has been found to possess high affinity for non-adrenoceptor sites labelled by [^{3}H]-*p*-aminoclonidine in the rat kidney (MacKinnon *et al.*, 1993) and by [^{3}H]-clonidine in the rabbit kidney and forebrain (Hamilton *et al.*, 1991). However, since these latter binding sites are labelled with relatively low affinity their pharmacological significance is questionable. I_2-binding sites, which are generally identified by use of [^{3}H]-idazoxan, are present in the kidney of all species in which they have been looked for, including man (Lachaud *et al.*, 1992) and dogs (Evans & Haynes, 1994). It has been argued that these receptive sites could inhibit sodium reabsorption, since they have been localized to the proximal tubular basolateral membranes in rabbits (Coupry *et al.*, 1989) and a number of drugs with high affinity for I_2-binding sites inhibit Na^+/H^+ exchange by a non-adrenoceptor mechanism (Frelin *et al.*, 1986; Bidet *et al.*, 1990). There is also some evidence from studies in anaesthetized dogs suggesting that guanabenz is natriuretic (Strandhoy *et al.*, 1982; 1983). Moreover, clinical studies of the acute and chronic effects of guanabenz suggest that it lacks the salt retaining effects of more selective α_2-adrenoceptor agonists such as α-methyldopa and clonidine (Walker *et al.*, 1981; Bauer, 1983; Gehr *et al.*, 1986).

We tested the effects of guanabenz on renal function, including renal sodium handling, under a range of experimental conditions. Despite elevating arterial pressure, guanabenz (10–100 μg kg^{-1}) did not increase sodium excretion either in dogs with intact autonomic reflexes or in ganglion-blocked dogs. Because a natriuretic effect of guanabenz could conceivably take some time to develop, we also tested the effects of infusing a low dose (5 μg kg^{-1} plus 0.2 μg kg^{-1} min^{-1}) over a 90 min period, which also had no effect on sodium excretion. We also reasoned that a natriuretic effect of activation of putative I_2-binding sites by guanabenz might be opposed by the antinatriuretic effects of the drug mediated by activation of tubular α_2-adrenoceptors (see Nord *et al.*, 1987). However, following blockade of α_2-adrenoceptors by 2-methoxyidazoxan (15 μg kg^{-1} plus 0.6 μg kg^{-1} min^{-1}), guanabenz (10–100 μg kg^{-1}) still had no natriuretic effect. We can be confident that this dose of 2-methoxyidazoxan provided significant antagonism at α_2-adrenoceptors, since it completely prevented the pressor, renal vasoconstrictor and polyuric effects of both guanabenz and rilmenidine in dogs with intact autonomic reflexes, and either prevented or significantly attenuated the effects of guanabenz and rilmenidine in ganglion-blocked dogs. Since 2-methoxyidazoxan has extremely low affinity for I_2-binding sites in the dog kidney (Evans & Haynes, 1994), so is unlikely to antagonize the effects of guanabenz at putative I_2-receptors, we conclude that α_2-adrenoceptors play a dominant role, and putative I_2-receptors play little or no role, in the effects of guanabenz on renal function in conscious dogs. Furthermore, our results provide no support for the notion that I_2-receptive sites in the dog kidney mediate natriuresis.

Rilmenidine was used in the present study as a tool to activate putative I_1-binding sites. Rilmenidine is an α_2-adrenoceptor agonist, but appears to have about 10 fold higher affinity for I_1-binding sites than it does for α_2-adrenoceptors (Ernsberger *et al.*, 1992). It also displays about 100 fold selectivity for I_1- over I_2-binding sites (Ernsberger *et al.*, 1992). Moreover, recent experiments have provided strong evidence to suggest that the acute blood pressure lowering effect of this drug is predominantly due to an interaction with putative I_1-receptors (Gomez *et al.*, 1991; Chan *et al.*, 1993; Sannajust & Head, 1994). I_1-like binding sites have been found in the brainstem; particularly in important vasomotor centres in the ventrolateral medulla, in rats, man, and other species (see Reis *et al.*, 1992), and it is these sites which are generally thought to mediate the acute blood pressure lowering effects of drugs like rilmenidine and moxonidine (Gomez *et al.*, 1991; Haxhiu *et al.*, 1994). There is also evidence from studies in anaesthetized rats that activation of brain I_1-binding sites by rilmenidine leads to natriuresis secondary to an inhibition of sympathetic drive to the kidney (Kline & Cechetto, 1993; Penner & Smyth, 1994). I_1-binding sites have also been found in the kidneys of rats and rabbits (Ernsberger *et al.*, 1990; Hamilton *et al.*, 1991; MacKinnon *et al.*, 1993). It has been argued that activation of I_1-binding sites in the kidney and/or brain could be natriuretic, and so potentially contribute to the long term antihypertensive effects of drugs like rilmenidine and moxonidine, but the evidence for this comes exclusively from studies in anaesthetized rats (see Smyth *et al.*, 1992; Allan *et al.*, 1993; Penner & Smyth, 1994).

In previous studies in dogs (either conscious or anaesthetized), the effects on sodium excretion of intravascular (either intravenous or intrarenal) administration of α_2-adrenoceptor agonists such as clonidine and guanabenz have ranged from antinatriuresis to natriuresis. However, these studies are difficult to interpret since in all except one (Strandhoy *et al.*, 1983) no control experiments are reported (Marchand *et al.*, 1971; Chrysanthakopoulos & Lavender, 1975; Olsen, 1976; Strandhoy *et al.*, 1982). Our study clearly shows that intravenous administration of guanabenz, which is an α_2-adrenoceptor agonist with very low affinity for I_1-binding sites, causes a diuresis but not a natriuresis in conscious normally-hydrated dogs, regardless of whether their autonomic reflexes are intact, or blocked by administration of pentolinium. We would expect therefore, that any natriuretic effect of rilmenidine, mediated by activation of putative I_1-receptors in the brainstem or kidney, would be clearly seen under our experimental conditions. In contrast, we found that the effects of rilmenidine on renal function were indistinguishable from those of guanabenz, and were antagonized by co-administration of the selective α_2-adrenoceptor antagonist, 2-methoxyidazoxan. We therefore conclude that our observations provide no support for the hypothesis that putative I_1-receptors mediate a natriuretic influence in conscious, normally-hydrated dogs.

The above conclusion is at odds with the data provided from experiments in anaesthetized rats (Smyth *et al.*, 1992; Allan *et al.*, 1993; Penner & Smyth, 1994), which suggest that in this species putative I_1-receptors in the central nervous system and/or kidneys mediate a natriuretic influence. There are a number of possible explanations for this discrepancy; including (i) that anaesthesia and volume loading in the rat experiments (see Penner & Smyth, 1994) could promote a natriuretic effect of I_1-agents, possibly through changes in renal sympathetic drive (Matsukawa *et al.*, 1993) or intrarenal haemodynamic and tubular factors (Alberola *et al.*, 1992), (ii) that rilmenidine may affect renal sympathetic drive differently in dogs compared with rats and rabbits, in which it dose-dependently reduces renal sympathetic nerve activity (Kline & Ce-

chetto, 1993; Szabo *et al.*, 1993), or (iii) that unlike the rat in which I_1-binding sites appear to predominate over I_2-binding sites (Ernsberger *et al.*, 1990; MacKinnon *et al.*, 1993), the kidney of the dog may lack putative I_1-receptors (see Evans & Haynes, 1994). These issues require further study.

Effects of 2-methoxyidazoxan and idazoxan

We used 2-methoxyidazoxan to antagonize the effects of guanabenz and rilmenidine mediated by activation of α_2-adrenoceptors, since this agent has extremely low affinity for I_2-binding sites, including those in the kidney of the dog (Evans & Haynes, 1994). Moreover, it has been used by a number of groups to discriminate between the α_2-adrenoceptor-mediated and I-receptor mediated effects of centrally acting antihypertensive agents with high affinity for I_1-binding sites, such as clonidine, rilmenidine and moxonidine (Chan *et al.*, 1993; Szabo *et al.*, 1993; Sannajust & Head, 1994). We found that in ganglion-blocked dogs, 2-methoxyidazoxan had no effect on blood pressure at cumulative doses from 0.3 to 30 $\mu g\ kg^{-1}$, indicating that at these doses this agent has negligible agonist action at receptors mediating vasoconstriction, including α-adrenoceptors. In fact, the only discernible effect of 2-methoxyidazoxan in ganglion-blocked dogs was a moderate and transient antinatriuresis when a high dose (30 $\mu g\ kg^{-1}$) was given acutely. This effect was not seen in other experiments in which ascending doses of 2-methoxyidazoxan were administered (0.3–30 $\mu g\ kg^{-1}$), so is of dubious significance. In contrast, in dogs with intact autonomic nervous systems, a dose of 30 $\mu g\ kg^{-1}$ 2-methoxyidazoxan had profound effects, which included increasing arterial pressure, heart rate, haematocrit and plasma renin activity, and reducing sodium excretion. These observations suggest that 2-methoxyidazoxan increases sympathetic drive in conscious dogs, through an action in the central nervous system. Since this agent is a highly selective antagonist of α_2-adrenoceptors, we conclude that a tonically active endogenous α_2-adrenergic mechanism inhibits sympathetic drive in conscious dogs.

The effects of idazoxan (3–300 $\mu g\ kg^{-1}$) in ganglion-blocked dogs were very different to those of 2-methoxyidazoxan. Idazoxan dose-dependently increased arterial pressure. This effect appears not to be attributable to an agonist action at α_2-adrenoceptors, since (i) it was not antagonized by co-administration of 2-methoxyidazoxan at doses (0.3–30 $\mu g\ kg^{-1}$) that in other experiments dose-dependently antagonized the effects of guanabenz and rilmenidine, and (ii) it was not accompanied by the polyuria that in other experiments occured during infusin of guanabenz or rilmenidine. It is likely that the effects of idazoxan are attributable to an agonist effect at α_1-adrenoceptors, since previous studies have shown that its pressor effect in pithed rats is prevented by pretreatment with the α_1-adrenoceptor antagonist prazosin (Paciorek & Shepperson, 1983). Thus, idazoxan is not an appropriate tool to study the influence of putative imidazoline receptors on renal function in dogs.

Conclusions

The results of the present study were unequivocal, in that infusions of neither rilmenidine nor guanabenz increased sodium excretion in conscious dogs, regardless of whether their autonomic reflexes were intact or blocked, or whether or not α_2-adrenoceptors were blocked by administration of 2-methoxyidazoxan. It is also clear that all of the effects of these agents that we did observe, were predominantly if not completely mediated by activation of α_2-adrenoceptors. We therefore conclude that, at least in the conscious dog, putative I-receptors do not appear to contribute significantly to the renal effects of infusion of rilmenidine or guanabenz.

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