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SHORT COMMUNICATION

RENAL EFFECTS OF RILMENIDINE IN VOLUME-LOADED ANAESTHETIZED DOGS

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SUMMARY

1. In anaesthetized, fluid expanded rats rilmenidine has diuretic and natriuretic effects. There is strong evidence that the natriuresis is mediated by putative imidazoline receptors. In contrast, in conscious euvolaemic dogs rilmenidine has a diuretic effect that is entirely attributable to activation of α_2-adrenoceptors, but no natriuretic effect. To determine whether the effects of rilmenidine are truly species dependent, or merely dependent upon the influences of anaesthesia and volume status, we tested the effects of rilmenidine in pentobarbitone anaesthetized, volume-loaded dogs.

2. The effects of rilmenidine in anaesthetized, volume-loaded dogs were similar to those found in conscious euvolaemic dogs. Compared with vehicle treatment, levels of glomerular filtration rate, urine flow and haematocrit were increased following rilmenidine treatment. No effect of rilmenidine on sodium excretion was observed.

3. We conclude that the renal responses to rilmenidine in dogs are largely unaffected by anaesthesia and plasma volume status. In particular, the natriuretic effect seen in rats was not observed. We conclude that putative imidazoline receptors do not have a major influence on sodium excretion in dogs.

Key words: α_2-adrenoceptors, dog, imidazoline receptor, kidney, pentobarbitone anaesthesia, plasma volume expansion, renal blood flow, rilmenidine sodium excretion.

INTRODUCTION

There is increasing evidence that some so-called 'second generation' centrally acting antihypertensive agents, such as rilmenidine and moxonidine, lower arterial pressure chiefly by an action at putative I_1-imidazoline receptors rather than at α_2-adrenoceptors.[1–3] The predominant anatomical site of action for the acute blood pressure lowering effects of these compounds is almost certainly the brainstem, and in particular the rostral ventrolateral medulla.[1–3] However, recent studies have identified a further mechanism that could contribute to the long term antihypertensive effects of these agents; a natriuretic effect, mediated possibly via inhibition of renal sympathetic drive or through a direct effect on the kidney. Evidence in support of this notion, however, has come exclusively from experiments performed in pentobarbitone anaesthetized rats.[4–7] Therefore, we recently investigated the effects of rilmenidine on renal function in conscious, euvolaemic dogs.[8] In contrast to the findings in anaesthetized rats, we could find no evidence that rilmenidine was natriuretic. Furthermore the effects of rilmenidine were indistinguishable from those of guanabenz, an α_2-adrenoceptor agonist with very low affinity for I_1-binding sites,[1] and were similarly antagonized by the selective α_2-adrenoceptor antagonist 2-methoxyidazoxan.[8]

One possible explanation for these disparate findings is that putative imidazoline receptors influence sodium excretion in rats but not dogs. However, an alternative explanation is that the effects of rilmenidine on renal function are highly dependent upon the experimental conditions. Indeed the anaesthesia and volume loading induced in the rat experiments are likely to have had profound influences on sympathetic drive to the kidney[9] and intrinsic intrarenal haemodynamic and tubular factors controlling sodium reabsorption.[10] Furthermore, we have previously found in rabbits that the renal excretory response to another pharmacological intervention, blockade of nitric oxide synthesis, is profoundly altered by anaesthesia and is dependent on salt status.[11] More significantly, others have reported the results of studies in anaesthetized dogs showing that volume expansion promotes a natriuretic effect of the α_2-adrenoceptor agonist, guanabenz.[12] Therefore, in the present study we tested the renal effects of infusing rilmenidine in dogs under conditions of pentobarbitone anaesthesia and modest plasma volume expansion.

METHODS

All experimental procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes,[13] and were approved in advance by the Alfred Hospital/Baker Medical Research Institute Animal Experimentation Ethics Committee. Twelve male greyhound dogs were used, which weighed between 30 and 35 kg (mean 32) at the time of the experiment.

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Surgical preparation

The preparative surgery and post-operative care of the dogs has been described in detail elsewhere.[8,14] Briefly, via a left retroperitoneal incision the dogs were equipped with a flow probe (type 6SB or 5RB, Transonic Systems Inc., Ithaca, NY, USA) around the left renal artery, a catheter in the left renal artery, two catheters in the abdominal aorta, and two catheters in the inferior vena cava. The right kidney was removed via a right retroperitoneal incision. Over the next 3–5 months these dogs were then used in other experiments which involved chronic infusion of AngII intrarenally and intravenously.[15] However, all dogs had been free from experimental intervention for at least 2 weeks before the procedures described in this paper were performed. Furthermore, all dogs were normotensive (conscious recumbent mean arterial pressure <110 mmHg) and had plasma creatinine levels within the normal range for uninephrectomized dogs (1.0–2.1 mg/100 mL).

Experimental protocol

Each dog was anaesthetized with sodium pentobarbitone (30–40 mg/kg + 10 mg/kg per h), and following intubation, artificially respired with a tidal volume of 18 mL/kg at a rate of 16 breaths/min so that arterial P_{O_2} and P_{CO_2} were maintained between 86–140 and 30–37 mmHg, respectively. The experiments were performed on a heated table to maintain body temperature between 36 and 39°C. From the time of intubation until the completion of the experiment, an intravenous infusion of 0.45% w/v NaCl and 2.5% w/v dextrose was administered at a rate of 4 mL/min. During the preparative surgery the ureter was catheterized via a left flank incision (20–40 min duration). One litre of a polygeline/electrolyte solution (Haemaccel, Hoechst Australia Ltd, Melbourne, Australia) and 1 L of 0.9% w/v NaCl was then administered via an intravenous drip, over a period of 30–40 min. 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), and blood gases. Each dog was then administered (intravenous) bolus doses of [^{3}H]-inulin (1.1 MBq; New England Nuclear, Sydney, Australia) and PAH (75 mg) (Sigma Chemical Company, St Louis, MO, USA). The infusion of NaCl/dextrose was then supplemented with [^{3}H]-inulin (3.7 MBq/mL) and PAH (0.82 mg/mL).

Seventy-five minutes later the first of four (consecutive) 30 min experimental periods began. The urine produced during these 30 min clearance periods was collected, and blood samples (20 mL) were taken half-way through each clearance period for renal function measurements. Plasma volume was replaced whenever blood was collected, by intravenous administration of an equivalent volume of a polygeline/electrolyte solution (Haemaccel, Hoechst Australia Ltd, Melbourne, Australia). At the start of the second clearance period, the dogs were treated with either rilmenidine (50 μg/kg over 5 min followed by 2 μg/kg per min; $n = 6$) (Servier Laboratories, Paris, France) or its vehicle (5% w/v dextrose; 125 μL/kg over 5 min followed by 5 μL/kg per min; $n = 6$).

Analysis of urine and blood samples and measurement of haemodynamic and renal variables have been described in detail elsewhere.[8]

Statistical analysis

All data are expressed as the mean ± SEM. The statistical computer software package SYSTAT[16] was used for statistical analyses. The levels of the variables during the first experimental period were compared across the two treatment groups by unpaired *t*-test. The effects of rilmenidine were tested for, using repeated measures analysis of variance.[17] The interaction term between treatment and time, was used as the test statistic, and *P* values were conservatively adjusted for compound asymmetry using the Greenhouse-Geisser correction.[17]

RESULTS

During the first 30 min clearance period there were no systematic differences in the levels of any of the measured variables between the two groups of dogs ($P \geq 0.4$ in all cases; Fig. 1). Haematocrit during the first 30 min clearance period was also similar in the two groups of dogs, averaging 36.0 ± 1.0% ($P = 0.3$). Haematocrit was also measured in these dogs 1–7 days before the experiment, when it averaged 42.1 ± 1.1%. Thus, the nominal level of plasma volume expansion produced in our experiment was 17.3 ± 2.5% of the resting plasma volume of each animal.

Across the time-course of the experiment, compared with vehicle, rilmenidine increased glomerular filtration rate, urine flow and haematocrit ($P \leq 0.05$ in all cases; Fig. 1). Mean arterial pressure and fractional urine excretion also tended to be greater during rilmenidine-treatment compared with vehicle-treatment, although these differences were not statistically significant ($P = 0.1$ in both cases; Fig. 1). Rilmenidine had no consistent effect on renal blood flow or the absolute or fractional excretion of sodium ($P \geq 0.2$ in all cases; Fig. 1), or on heart rate, filtration fraction or fractional potassium excretion ($P \geq 0.2$ in all cases; data not shown).

DISCUSSION

In the present study we tested the effects of rilmenidine in pentobarbitone anaesthetized, plasma volume expanded dogs, in order to determine whether the renal responses to rilmenidine under these experimental conditions are different from those observed in conscious, euvolaemic dogs.[8] Our hypothesis was that anaesthesia and plasma volume expansion would promote a natriuretic effect of rilmenidine similar to that observed in anaesthetized rats.[7] Our major conclusion is that the renal response to rilmenidine in dogs is largely unaffected by pentobarbitone anaesthesia and plasma volume expansion. Therefore we further conclude that the renal response to rilmenidine in dogs is clearly different from that in rats, particularly in that the natriuretic effect observed in rats is absent in dogs.

In pentobarbitone anaesthetized, plasma volume expanded dogs, the chief effect of rilmenidine (50 μg/kg + 2 μg/kg per min) was to increase glomerular filtration rate. This increased glomerular filtration rate may have been secondary to increased arterial pressure, even though this latter effect was not statistically significant ($P = 0.1$). A major contribution of the sympatholytic effect of rilmenidine to the increased filtration seems unlikely, as in conscious dogs ganglion blockade reduces rather than increases glomerular filtration rate, even though mean arterial pressure is unchanged.[8] An increase in urine flow was also observed, but this appeared to be primarily due to the increased filtered load as no consistent change in fractional urine excretion was observed. Rilmenidine administration was also followed by a gradual rise in haematocrit, but no consistent effects on heart rate, renal blood flow or sodium excretion were observed.

This profile of effects is very similar to that observed in conscious euvolaemic dogs. In conscious dogs under conditions of ganglion blockade, the same dose of rilmenidine increased arterial pressure, glomerular filtration rate and urine flow, without affecting renal blood flow or sodium excretion.[8] In conscious dogs with intact autonomic reflexes, the profile of renal effects of rilmenidine was slightly different, in that the pressor effect was blunted compared with ganglion blocked dogs, there was no change in glomerular filtration rate, and

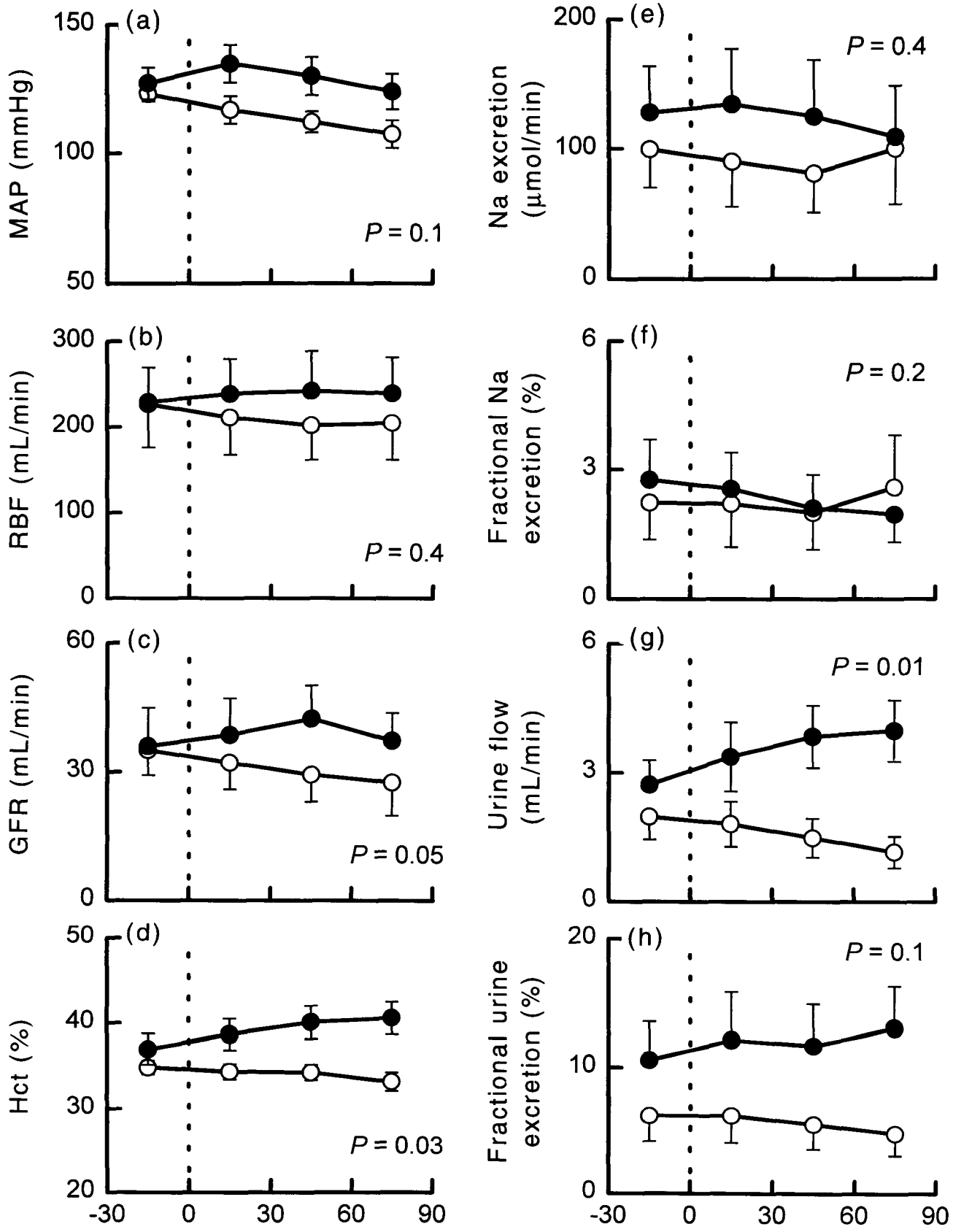
(a)
MAP (mmHg)
150
100
50
P = 0.1
(b)
RBF (mL/min)
300
200
100
0
P = 0.4
(c)
GFR (mL/min)
60
30
0
P = 0.05
(d)
Hct (%)
50
40
30
20
P = 0.03
(e)
Na excretion (μmol/min)
200
100
0
P = 0.4
(f)
Fractional Na excretion (%)
6
3
0
P = 0.2
(g)
Urine flow (mL/min)
6
3
0
P = 0.01
(h)
Fractional urine excretion (%)
20
10
0
P = 0.1
-30
0
30
60
90
Time from administration (min)

Fig. 1. Effects of rilmenidine (●; 50 μg/kg + 2 μg/kg per min, i.v.) and its vehicle (○; 5% w/v dextrose, 125 μL/kg over 5 min followed by 5 μL/kg per min) on (a) mean arterial pressure (MAP), (b) renal blood flow (RBF), (c) glomerular filtration rate (GFR), (d) haematocrit (Hct), (e) sodium excretion, (f) fractional sodium excretion, (g) urine flow and (h) fractional urine excretion in plasma volume-loaded, anaesthetized dogs. Each point shows the mean ± SEM of six observations. The *P* values represent the outcomes of repeated measures analyses of variance testing for interactions between treatment (rilmenidine or vehicle) and time.

renal blood flow and sodium excretion were dose-dependently reduced.[8]

I_1-binding sites have been identified in rat kidney and brain (see[18]), and natriuretic responses appear to be mediated by activation of putative I_1-receptors in both of these regions.[4–7,18] We have been unable to identify I_1-binding sites in dog kidney,[19] and have no information regarding the existence of these binding sites in the dog central nervous system. We cannot determine with any certainty, therefore, whether our finding that rilmenidine is not natriuretic in dogs reflects a lack of putative I_1-receptors in dog brainstem and/or kidney, an interspecies difference between the effects mediated by putative I_1-receptors in these two species, or that the doses of rilmenidine we used did not sufficiently activate I_1-receptors (in either the brain or the kidney) under our experimental conditions. However, the latter possibility seems unlikely given that we have not observed a natriuretic effect of rilmenidine under any of the experimental conditions we have employed. In our previous study[8] we tested the effects of a range of doses of rilmenidine, so we can be confident that we covered the potential natriuretic dose-range. We have also tested the effects of rilmenidine under conditions of blockade of the confounding, and indeed possibly antinatriuretic influence of α_2-adrenoceptor activation.[8] We can also be confident that the conditions of the present study were not already maximally natriuretic, which could obscure a potential natriuretic effect of rilmenidine. Baseline fractional sodium excretion averaged 2.5% in the volume-loaded anaesthetized dogs used in the present study, considerably less than that observed in (euvolaemic) conscious ganglion blocked dogs (4.0%), though also somewhat greater than that observed in (euvolaemic) conscious dogs with intact autonomic reflexes (1.7%).[8]

There is now considerable evidence that in rats putative I_1-receptors influence sodium handling.[4–7] Taken together, the results of our previous study of conscious dogs,[8] and the results of the present study, suggest that putative I_1-receptors do not have a major influence on renal sodium handling in dogs.

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