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Rilmenidine (S 3341) and the sympatho-adrenal system: adrenoreceptors, plasma and adrenal catecholamines in dogs

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1 The effects of rilmenidine, a new alpha$_2$-adrenoreceptor agonist with antihypertensive properties, were investigated on plasma catecholamines, blood cell adrenoreceptors and adrenal medullary function.

2 In conscious sino-aortic denervated (SAD) dogs, rilmenidine (1 mg kg^{-1} orally for 2 weeks) significantly reduced both blood pressure and heart rate when compared with placebo treatment. The drug decreased plasma noradrenaline and adrenaline levels and corrected the decrease in leucocyte beta-adrenoreceptors observed in placebo-treated SAD dogs. There was no change in platelet alpha$_2$-adrenoreceptors.

3 In anaesthetized normotensive dogs, rilmenidine (0.1 and 0.3 mg kg^{-1} i.v.) induced a dose-dependent decrease in both cardiovascular parameters (blood pressure and heart rate) and catecholamine release from the adrenal medulla.

4 The present study shows that rilmenidine decreases sympathetic tone mainly by an action on the adrenal medulla. In addition, its ability to lower blood pressure in SAD dogs, i.e. a model of hypertension in which high sympathetic tone is present, indicates that rilmenidine may also depress other parts of the sympathetic nervous system.

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Introduction

Rilmenidine (S 3341) ([(N-dicyclopropylmethyl)-amino]-2Δ2-oxazoline) a new hypotensive agent possesses more selectivity for alpha$_2$-adrenoreceptors than clonidine (Dausse, Guicheney & Meyer, 1981; Guicheney, Dausse & Meyer, 1981; Laubie *et al.*, 1985). Moreover, clinical studies have demonstrated its antihypertensive properties in man (Ostermann, Brisgand, Schmitt & Filastre, 1988) with significantly less sedation than clonidine (Weerasuriya, Shaw & Turner, 1984). The aim of the present work in dogs was to study the antihypertensive effect of rilmenidine in dogs and to examine the possible involvement of the sympathetic nervous system in this effect. Thus, two different *in vivo* protocols were used: first, chronic, neurogenic hypertension in conscious sinoaortic denervated (SAD) dogs, a model characterized by high sympathetic tone (Damase, Montastruc, Montastruc & Valet, 1987) and secondly catecholamine release from the adrenal medulla in anaesthetized dogs (Anglade *et al.*, 1987). For this purpose, the effects of rilmenidine on plasma and adrenal catecholamine levels and circulating blood cell adrenoreceptors were measured.

Methods

Protocol 1: Study of rilmenidine in conscious SAD dogs

Sinoaortic denervation (SAD). Seven mongrel dogs of either sex weighing 20 to 25 kg were made hypertensive by SAD as previously described (Damase *et al.*, 1987). Briefly, carotid and aortic nerves were cut under chloralose anaesthesia (80 mg kg^{-1} i.v.) during two successive procedures, one at time 0 and the second one 7 weeks later. During the surgery, care was taken to keep intact both vagal and sympathetic fibres in the vagus. The effectiveness of baroreceptor denervation was checked by the failure of phenylephrine (0.1, 1 and 10 μg kg^{-1} i.v.) to induce bradycardia and nitroglycerin (1, 3, 10 and 30 μg kg^{-1} i.v.) to induce tachycardia (Damase *et al.*, 1987).

Leucocyte and platelet membrane preparations. Leucocytes and platelets were isolated from fresh blood collected into EDTA 2% according to the method of Boyum (1976). 5 ml of blood was diluted with an equal volume of buffer (NaCl 140 mM 9 vol; TRIS 145 mM, KCl 5.4 mM, $MgCl_2$ 0.98 mM, $CaCl_2$ 0.05 mM, D-glucose 5.5 mM 1 vol) and carefully layered into 10 ml of Ficoll-Paque (d=1077). Tubes were centrifuged at 400 ***g*** for 30 min at 20°C. After careful removal of the upper layer containing the platelets, the leucocyte band was washed twice with three volumes of buffer and centrifuged at 60 ***g*** for 10 min at 20°C. The plasma/platelet layer was washed twice with three volumes of buffer and centrifuged at 5000 ***g*** for 10 min at 20°C. Blood cells (leucocytes or platelets) were homogenized at 4°C in a potter apparatus with a teflon-tipped pestle in 8 ml of a hypotonic buffer (5 mM TRIS-HCl, 5 mM EDTA, pH 7.5). The membrane preparations were centrifuged at 39000 ***g*** for 15 min at 4°C, then diluted in 1 ml binding buffer (50 mM TRIS HCl, 10 mM $MgCl_2$, 120 mM NaCl, pH 7.5 and finally stored at −80°C; assays were usually performed within 1–2 days.

Binding studies. The membranes were assayed for adrenoreceptors by the radioligand binding technique as previously described (Williams, Snyderman & Lefkowitz, 1976; Villeneuve, Berlan, Lafontan & Montastruc, 1985). Briefly, the radioligands used for adrenoreceptor identification were antagonists, total binding was determined by incubating 50 μl aliquot of the resuspended membrane preparation (50 μg protein assayed by the method of Lowry, Rosebrough, Farr & Randall, 1951) with (^{125}I)-cyanopindolol or (^{3}H)-yohimbine, in a total volume of 200 μl of binding buffer. Specific binding was defined as the difference between total and non-specific binding determined in parallel assays but containing an excess (10 μM) of either propranolol or phentolamine. The final concentration of radioligand ranged from 10 to 300 pM for (^{125}I)-cyanopindolol and 0.2 to 15 nM for (^{3}H)-yohimbine. Incubations were carried out for 40 min at 37°C with (^{125}I)-cyanopindolol and 20 min at 25°C with (^{3}H)-yohimbine under constant shaking. Samples were filtered through fibreglass filters (Whatman GF/C) placed on a Nunc cell harvester unit. Filters were washed with 10

ml of cold binding buffer and the trapped radioactivity counted in a Packard spectrometer with an efficiency of 35% for ^{3}H and 75% for ^{125}I. Specific binding ranged from 80–90% for (^{3}-H)-yohimbine and 60–70% for (^{125}I)-cyanopindolol at concentrations of twice the Kd (affinity constant). The number of binding sites (Bmax) and Kd were calculated with a computer assisted analysis of binding at saturation and according to Scatchard analysis.

General procedure and drug administration. Rilmenidine (1 mg kg^{-1} orally for 15 days given at 0700 h) or placebo (lactose) were administered one month after SAD surgery according to a double-blind cross-over randomized protocol in the seven SAD hypertensive dogs with a one week wash-out interval. At the end of each treatment period, all physiological and biochemical measurements were made one hour after the last oral administration of either placebo or rilmenidine in dogs deprived for food but not water for 12 h. This time was determined according to preliminary studies (not shown) indicating that the maximal hypotensive effect occured 60 min after oral administration.

Protocol 2: Study of rilmenidine on adrenal medulla in anaesthetized dogs

General procedure. Eighteen mongrel dogs of either sex weighing 14 to 20 kg were anaesthetized with alpha-chloralose (80 mg kg^{-1} i.v.), curarized with gallamine (2 mg kg^{-1} i.v.) and artificially ventilated with a Palmer Ideal pump (insufflated air volume: 15 ml kg^{-1} with frequency of 16 min^{-1}). Fully adequate anaesthesia was maintained by an injection of 15–20 mg of chloralose each hour. Body temperature of the animals was maintained at a constant level around 38°C and arterial pH monitored using a Methrohm pH meter.

Adrenal venous blood sampling. Adrenal venous blood sampling was made according to our previously described technic (Anglade *et al.*, 1987). Briefly, after median laparatomy, the right adrenal vein was dissected and its collaterals occluded. The animal was heparinized (500 U kg^{-1} every 2 h) and the adrenal vein occluded at the level of its junction with the inferior vena cava. The other end was cannulated to enable blood sampling. When not being sampled, adrenal venous blood was directly returned to the femoral vein via a cannula. Blood sampling started 25 min after adrenal vein cannulation. Each blood sample (5 ml) was collected in a tube containing anticoagulant heparin (0.1%) and immediately frozen. Plasma was immediately separated by centrifugation and stored below −80°C. Adrenal plasma flow rate (ml kg^{-1} min^{-1}) was measured and catecholamine output (adrenaline and noradrenaline) expressed as ng kg^{-1} min^{-1} of the base.

Under our experimental conditions (full anaesthesia and curarization with gallamine), blood pressure and heart rate remained stable during the whole experiment. Catecholamine levels, especially noradrenaline, could remain stable or even increase although arterial pH (7.42), body temperature (38°C) and a haematocrit (38) remained constant. For this reason 21 min was chosen for the end of the experimental design. Gallamine prevents the variations in carotid arterial pressure induced by respiratory irregularities without inducing ganglioplegy Sumikawa, Kashimoto, Isumi, Yoshikawa & Amakata (1979) claimed that it does not alter the secretion rate of adrenaline from the gland.

Drug administration. Rilmenidine (0.1 mg kg^{-1} or 0.3 mg kg^{-1} i.v. dissolved in saline) or saline alone were given according to a randomized protocol.

Measurement of Cardiovascular parameters

Systolic and diastolic blood pressures were recorded by means of a catheter introduced into the abdominal aorta via the left femoral artery according to the method of Sedlinger (1953) and connected to a Gould P231D transducer on one channel of a Honeywell recorder. Heart rate was obtained using a heart period (pulse interval) meter triggered by the electrocardiogram. Protocol 1 was performed in conscious dogs whereas protocol 2 under chloralose anaesthesia.

Catecholamine assays

Plasma or adrenal catecholamines (adrenaline and noradrenaline expressed in term of the bases) were measured by high pressure liquid chromatography using electrochemical detection (Valet *et al.*, 1988a). Blood samples were obtained from femoral artery for protocol 1 and adrenal vein for protocol 2.

Statistical analysis

Results are presented as mean values ± SEM. For protocol 1, significance was estimated by use of a cross-over ANOVA test comparing placebo- and rilmenidine-treated dogs and for protocol 2 by a two-way analysis of variance with repeated measures on time followed by a Newman-Keul test. P values higher than 0.05 were not considered to be significant.

Results

Protocol 1: Effects of rilmenidine in conscious SAD dogs

As previously reported (Damase *et al.*, 1987), SAD induced sustained increases in systolic and diastolic blood pressures and heart rate and an increase in plasma catecholamine levels. Simultaneously, platelet alpha$_2$ and leucocyte beta$_2$-adrenoreceptors of circulating blood cells (Bmax) significantly decreased (Table 1). There was no change in affinity constant (Kd).

Cardiovascular parameters. Placebo treatment failed to change the values of blood pressure in SAD hypertensive dogs. By contrast, rilmenidine treatment significantly decreased systolic (−46%; $P<0.001$), diastolic (−58%; $P<0.01$) blood pressures and heart rate (−46%; $P<0.001$) when compared with placebo, the values of which reaching pre-SAD values (normotensive dogs). There was no significant difference between pre-SAD (normotensive dogs) and rilmenidine treated SAD dogs (Table 1).

Plasma catecholamine levels. Placebo administration did not significantly modify the values of plasma catecholamine levels in SAD hypertensive dogs. Rilmenidine significantly decreased plasma levels of noradrenaline (−54%; $P<0.05$) and adrenaline (−57%; $P<0.05$) when compared with placebo (Table 1).

Platelet and leucocyte adrenoreceptors. Placebo administration failed to change the Bmax and Kd values of platelet alpha$_2$ and leucocyte beta-adrenoreceptors. In contrast, rilmenidine significantly increased the Bmax

Table 1. Effects of rilmenidine (1 mg kg^{-1} orally for 15 days) on systolic and diastolic blood pressures, heart rate noradrenaline and adrenaline plasma levels, platelet alpha$_2$-adrenoreceptor number (Bmax: [3H] YOH leucocyte beta$_2$-adrenoreceptor number (Bmax: [125I] CYP, in conscious dogs

	Normotensive dogs	SAD dogs	SAD dogs +placebo	SAD dogs +rilmenidine
Systolic blood pressure (mm Hg)	145 ± 4	205 ± 7***	212 ± 6	114 ± 5***
Diastolic blood pressure (mm Hg)	73 ± 3	109 ± 11***	117 ± 8	49 ± 4**
Heart rate (beats min^{-1})	87 ± 16	159 ± 9***	163 ± 7	88 ± 4***
Noradrenaline (pg m^{-1})	237 ± 33	1014 ± 236*	676 ± 101	304 ± 50*
Adrenaline (pg m^{-1})	146 ± 33	641 ± 146*	348 ± 74	146 ± 54*
[3H] YOH (fmol mg protein^{-1})	198 ± 12	133 ± 11*	145 ± 22	112 ± 10^{ns}
[125I] CYP (fmol mg protein^{-1})	48 ± 2	19 ± 4**	26 ± 4	57 ± 3**

Data are presented as mean values ± SEM. Statistical analysis, using Student's t-test for paired comparison, compares the same seven dogs before (normotensive dogs: column 1) and one month after SAD (SAD dogs: column 2). Cross-over ANOVA test was used for comparison between column 3 (SAD dogs+placebo) and column 4 (SAD dogs+rilmenidine). *$P<0.05$, **$P<0.01$, ***$P<0.001$.

(+54%; $P<0.01$) of leucocyte beta-adrenoreceptors, the values of which returning to values observed before SAD (normotensive dogs). Bmax values of platelet alpha$_2$-adrenoreceptors remained unchanged after rilmenidine and not significantly different from placebo treated SAD dogs (Table 1). There was no change in Kd.

Protocol 2: Effects of rilmenidine on adrenal medulla in anaesthetized dogs

Catecholamine release from the adrenal gland. Three groups of dogs were used: the first one (control group) received saline as a sham injection whereas others were treated with 0.1 or 0.3 mg kg^{-1} i.v. rilmenidine respectively. In these 18 dogs, catecholamine release as well as adrenal plasma flow rate were measured at time 0 (i.e. just before drug or saline injection) and 7, 14 and 21 min after drug administration. These times were chosen according to both preliminary studies (not shown) and the results of our study of clonidine (Anglade *et al.*, 1987).

In the control group ($n=6$), mean values of adrenaline and noradrenaline were respectively 10.5 ± 1.6 and 3.9 ± 1.3 ng kg^{-1} min^{-1} at time 0. Adrenaline levels slightly increased during the experiment whereas the changes in noradrenaline levels were more marked (in some but not all the animals): thus, for example, at time 14 min, the mean percentage of increase were respectively 4.8 ± 13.9 and 51.5 ± 45.1 for adrenaline and noradrenaline respectively when compared with basal values. But, these changes were not significant. Simultaneously, adrenal plasma flow rate showed a slight, constant but significant decrease: at time 0, mean flow rate was 0.256 ± 0.016 ml kg^{-1} min^{-1}, and at time 14 min, the mean percentage of decrease was 5.6 ± 1.7 versus time 0 ($P<0.001$).

In dogs treated with rilmenidine, mean values of adrenaline and noradrenaline were respectively 11.9 ± 2.3 and 3.5 ± 0.8 ng kg^{-1} min^{-1} for the 5 animals receiving 0.1 mg kg^{-1} and 15.4 ± 2.6 and 4.3 ± 0.7 ng kg^{-1} min^{-1} for the 7 animals receiving 0.3 mg kg^{-1} at time 0. Adrenal plasma flow rate was respectively 0.231 ± 0.028 and 0.228 ± 0.012 ml kg^{-1} min^{-1} in these two groups. Rilmenidine induced a dose-dependent decrease in catecholamine (adrenaline as well as noradrenaline) release from the gland at 7 min (data not shown), 14 min (Fig. 1) and 21 min (data not shown) after injection when compared with control groups. Figure 1 also shows changes in adrenal plasma flow rate. We note that adrenal plasma flow rate slightly decreased in the groups: however, the decrease in rilmenidine-treated groups was not statistically different from that observed in control animals.

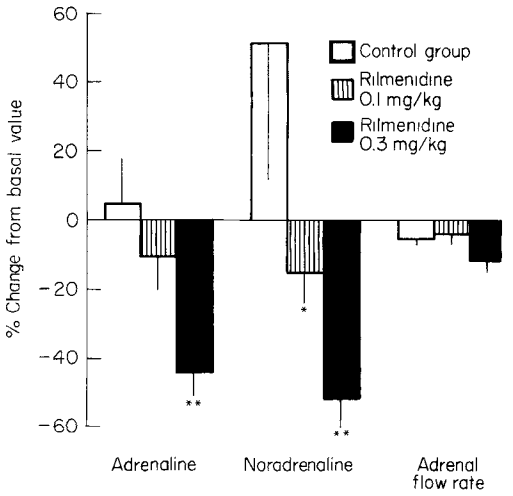

Fig. 1. Effects of rilmenidine on catecholamine release from the adrenal gland and adrenal plasma flow rate measured 14 min after 0.1 (hatched columns) and 0.3 (solid columns) mg kg^{-1} i.v. rilmenidine administration. Mean values ±SEM of percentage change with respect to basal values. *$P<0.05$, **$P<0.01$ when compared to control group (open column) using a Newman Keuls test.

Cardiovascular parameters. In control groups, cardiovascular parameters remained constant. Resting systolic, diastolic blood pressures and heart rate were respectively 149.4 ± 6.4 mmHg, 109.4 ± 5.9 mmHg, 155.8 ± 8.6 beats min^{-1} (in 0.1 mg kg^{-1} group), 151.4 ± 9.1 mmHg 105.1 ± 5.9 mmHg, 154.3 ± 6.6 beats/min (in 0.3 mg kg^{-1} group). Rilmenidine decreased blood pressure: however, a significant level was only reached for 0.3 mg kg^{-1} and after 14 min (Fig. 2). It was also observed at time 21 min (data not shown). In fact, rilmenidine first

induced a pressor response $+4.3 \pm 8.4$ and $+7.8 \pm 5.9$ (Δmm Hg, for systolic and diastolic blood pressure respectively) at the 7th min following injection. This effect is due to the postsynaptic alpha$_2$-adrenoreceptor vasoconstrictor properties of the drug which delays the appearance of the hypotensive effect of rilmenidine (Laubie *et al.*, 1985; Van Zwieten *et al.*, 1986). Simultaneously, heart rate significantly decreased whatever the dose and the time of observation (Fig. 2).

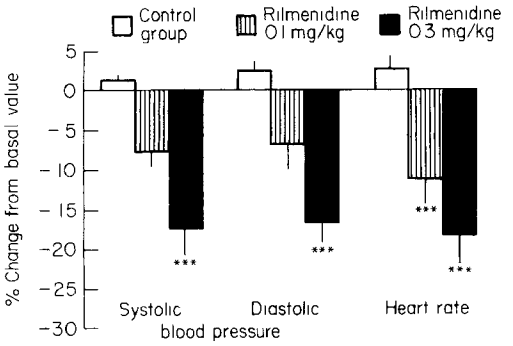

Fig. 2. Effects of rilmenidine on systolic and diastolic blood pressures and heart rate measured 14 min after 0.1 (hatched columns) and 0.3 (solid columns) mg kg^{-1} i.v. rilmenidine administration. Mean values $\pm$SEM of percentage change with respect to basal values. ***$P<0.001$ when compared to control group (open column) using a Newman Keul test.

Discussion

The present study investigates the effects of rilmenidine on the sympathetic nervous system. The involvement of the sympathetic nervous system was clearly demonstrated using the original model of chronic SAD hypertensive dogs. This experimental model appears to be a suitable one to investigate the effects of antihypertensive agents since it is characterized by a high sympathetic activity, as indicated by the increased levels in plasma catecholamines. Under those experimental conditions, rilmenidine significantly corrected the level of high blood pressure and also reduced heart rate, whereas placebo treatment did not. As far as we know, few data are available on the effects of chronic treatment with rilmenidine on experimental hypertension: Van Zwieten *et al.* (1986) showed that during a 12-day continuous subcutaneous infusion, rilmenidine reduced blood pressure and heart rate in SH rats.

Simultaneously, plasma levels of noradrenaline as well as adrenaline decreased in SAD dogs after rilmenidine when compared with placebo. This conclusion agrees with the data obtained in SH rats by Koenig-Berard *et al.* (1988).

It is interesting to underline that these variations in plasma catecholamines are accompanied by striking changes in adrenoreceptors numbers. As previously reported (Damase *et al.*, 1987), chronic SAD hypertensive dogs exhibited a lower number of leucocyte beta-adrenoreceptors (and to a lesser extent platelet alpha$_2$-adrenoreceptors) than normotensive animals. Rilmenidine treatment corrected these alterations in beta-adrenoreceptors: leucocyte beta-adrenoreceptors returned to pre-SAD values (i.e. normal values). In contrast, platelet alpha$_2$-adrenoreceptors did not vary after rilmenidine.

Other results and the present data clearly show that leucocyte beta (but not platelet alpha$_2$-adrenoreceptors) are regulated by the levels of endogenous catecholamines. In a previous study in patients with phaeochromocytoma, we found a significant decrease in leucocyte beta- but not in platelet alpha$_2$-adrenoreceptors (Valet *et al.*, 1988b). Moreover, in normotensive dogs neither adrenaline nor clonidine modified the number of platelet alpha$_2$-adrenoreceptors (Villeneuve *et al.*, 1985). This observation agrees with the conclusion of the review of Mahan, McKernan & Insel (1987) and suggests that still unknown mechanisms down-regulate platelet alpha$_2$-adrenoreceptors.

As far as we know, the effects of rilmenidine on adrenaline release from the adrenal gland have never been studied in the anaesthetized and curarized dog. Our present data clearly indicate that this drug reduces adrenaline as well as noradrenaline release from the adrenal medulla in dogs. The rilmenidine-induced fall in adrenal plasma flow rate cannot explain the inhibitory effect of the drug on adrenal secretion since the decrease in plasma flow rate was not statistically

different from that observed in control animals.

If these effects of rilmenidine are compared with those of the alpha$_2$-agonist clonidine, both drugs decrease adrenaline release from the gland (for example see Anglade *et al.*, 1987). For clonidine, most of the data from the literature indicate an exclusive or preponderant central mechanism of action (Togashi, 1983; Anglade *et al.*, 1987; Gaillard, Tran, Rostin, Salvayre & Montastruc, 1987). However, the respective contributions of a central or peripheral component to the decrease in catecholamine release from the gland in the effect of rilmenidine remains unknown. According to Lhoste *et al.* (1983), who showed a consistent decrease in plasma noradrenaline and to a lesser extent in plasma adrenaline (chosen as an index of adrenal medulla activity), the peripheral component could play an important role.

In conclusion, the present study shows that rilmenidine decreases sympathetic tone mainly by an action on the adrenal medulla. In addition, its ability to lower blood pressure in a model of hypertension in which high sympathetic tone is present indicates that rilmenidine may also depress other parts of the sympathetic nervous system.

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