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Interactions of the cardiac chronotropic effects of rilmenidine with the autonomic nervous system in conscious dogs: comparison with clonidine

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1 The cardiac chronotropic effects of rilmenidine ($10-100\,\mu g\,kg^{-1}$) and clonidine ($1-10\,\mu g\,kg^{-1}$) were studied in conscious dogs with chronic atrioventricular block.

2 Rilmenidine and clonidine initially (< 3 min) decreased atrial rate, although the effect was not related to dose. More lastingly, ventricular rate was decreased in a dose-related manner (ratio, 1:21). Rilmenidine lowered mean blood pressure only at $100\,\mu g\,kg^{-1}$, while clonidine had the same effect at doses of $5\,\mu g\,kg^{-1}$ upward (ratio, 1:15).

3 When administered after atropine and pindolol, rilmenidine ($50\,\mu g\,kg^{-1}$) produced a decrease in atrial rate, with an identical intensity but longer duration than under basal conditions. When clonidine ($2.5\,\mu g\,kg^{-1}$) was given after atropine, no chronotropic atrial effect was observed. However, when clonidine ($2.5\,\mu g\,kg^{-1}$) was given after pindolol, it produced a decrease in atrial rate that was more marked, both in intensity and duration, than under basal conditions. After phenoxybenzamine, rilmenidine decreased atrial rate with a more marked and lasting effect than observed under basal conditions. Clonidine produced a bradycardic atrial effect identical to the basal effect. After yohimbine, rilmenidine and clonidine decreased atrial rate with an intensity similar to that under basal conditions, although the time course was totally different.

4 When given after atropine, rilmenidine ($50\,\mu g\,kg^{-1}$) and clonidine ($2.5\,\mu g\,kg^{-1}$) decreased ventricular rate as under basal conditions, whereas after pindolol and phenoxybenzamine, both drugs decreased ventricular rate less markedly than under basal conditions, both in intensity and duration. After yohimbine, rilmenidine and clonidine produced no chronotropic ventricular effect.

5 These results show that (a) the initial atrial bradycardia caused by rilmenidine results from both a decrease in sympathetic tone and an increase in cholinergic activity; while the effect of clonidine is caused mainly by the enhancement of cholinergic activity. For both drugs, α_2-adrenoceptors are involved at least in the initiation of the effect; (b) the very short duration of atrial bradycardia may result from reflex buffering in response to ventricular bradycardia. This buffering is less effective when heart rate was high; and (c) the ventricular bradycardia caused by both drugs is mainly the result of a decrease in sympathetic tone in response to the stimulation of α_2-adrenoceptors. The results also suggest that negative chronotropic postsynaptic α_2-adrenoceptors could be involved in the ventricular bradycardia.

Introduction

Rilmenidine, (2-(dicyclopropylmethyl)amino-2-oxazoline), is an antihypertensive agent known to bind more selectively to imidazoline-preferring receptors than to α_2-adrenoceptors (Bricca *et al.*, 1989, 1994; Gomez, Ernsberger, Feinland & Reis, 1991) and to α_2- more selectively than to α_1-adrenoceptors (Van Zwieten, 1988). Its blood pressure lowering effects have been demonstrated in both animals and humans (Beau *et al.*, 1988; Koenig-Bérard *et al.*, 1988; Feldman *et al.*, 1990; Gomez *et al.*, 1991; Laurent & Safar, 1992). Rilmenidine has also been shown to either decrease (Laubie *et al.*, 1985; Koenig-Bérard *et al.*, 1988; Boucher *et al.*, 1994c; Sannajust & Head, 1994) or not modify (Dollery *et al.*, 1988; Spiers,

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Harron & Wilson, 1990; Tonet *et al.*, 1991) heart rate depending on the dose and/or route and mode of administration used. To our knowledge, no extensive study of the cardiac chronotropic effects of rilmenidine have been carried out in conscious dogs.

We studied the cardiac chronotropic effects of rilmenidine as a function of dose in conscious dogs with chronic atrioventricular (AV) block. The effects were compared with those of clonidine, the well-known antihypertensive α_2-adrenoceptor agonist, that has already been shown to decrease atrial and ventricular rates in conscious AV-blocked dogs (Duchêne-Marullaz *et al.*, 1974; Boucher, Dubray & Duchêne-Marullaz, 1982a). This experimental model has marked vagal tone associated with weak adrenergic tone at the atrial level, and marked adrenergic tone associated with weak vagal tone at the ventricular level (Robinson, Farr & Grupp, 1973; Duchêne-Marullaz *et al.*, 1975; Reynolds & Di Salvo, 1978; Boucher, Duchêne-Marullaz & Lavarenne, 1979; Boucher & Duchêne-Marullaz, 1980). Hence, direct and reflex chronotropic effects can be readily distinguished. In addition, we focused especially on the mechanism(s) of the chronotropic effects of rilmenidine, and in particular on interactions with the autonomic nervous system.

Methods

We studied six mongrel dogs of either sex, weighing between 15 and 23 kg. They were housed in individual cages in a large colony room with food and water continuously available in their home cages. The study conformed to the NIH *Guidelines for Care and Use of Laboratory Animals*.

Surgical preparation and instrumentation

AV block had been induced in the six dogs at least 2 months before the study, long enough for atrial and ventricular rates to stabilize (Boucher, Dubray & Duchêne-Marullaz, 1982b). AV block was produced under pentobarbital anaesthesia by crushing the His bundle with forceps introduced through the open right atrium during temporary occlusion of the venae cavae (modified Fredericq's technique (Boucher & Duchêne-Marullaz, 1985)). Four dogs were also fitted with a catheter for long-term measurement of blood pressure (BP), which was inserted into the left omocervical artery and connected to a valve fixed on the neck.

Measurements

ECG and BP were monitored by using a Cardiopan III T instrument (Massiot-Philips, Clermont-Ferrand, France) and a Statham P23 Gb transducer (Gould, Paris, France) connected to the arterial valve and linked to the recorder through a pressure module. During recording, the dogs, which had been thoroughly familiarized previously with the experimental conditions, were placed on a table and lightly restrained. One or two microcatheter(s) were fitted before each test, one in the cephalic vein and the other in a branch of the saphenous vein, to allow painless drug administration and blood sampling, respectively.

Protocol

Rilmenidine (as the dihydrogen phosphate) was administered intravenously at doses of 10, 25, 50, and 100 $\mu g\,kg^{-1}$, and clonidine (as the hydrochloride) at doses of 1, 2.5, 5, and 10 $\mu g\,kg^{-1}$. Sufficiently high doses of rilmenidine were chosen to produce significant chronotropic effects throughout the dose range; and those of clonidine to induce almost identical effects. The 50 $\mu g\,kg^{-1}$ dose of rilmenidine and the 2.5 $\mu g\,kg^{-1}$ dose of clonidine were repeated after blockade of (a) muscarinic cholinoceptors with atropine sulphate (200 $\mu g\,kg^{-1}\,h^{-1}$ i.v.); (b) β-adrenoceptors with pindolol base (0.5 $mg\,kg^{-1}$ i.v.); and (c) α-adrenoceptors with either phenoxybenzamine hydrochloride (5 $mg\,kg^{-1}$ i.v.) or yohimbine hydrochloride (0.5 $mg\,kg^{-1}$ i.v.).

The muscarinic receptors were blocked by using an infusion of atropine rather than a single injection, to maintain a stable atrial cardioacceleration throughout the experiments. Pindolol was used, since in this model a large dose could be administered, thus inducing a very high degree of β-adrenoceptor blockade (as verified by antagonism of isoprenaline hydrochloride (1 $\mu g\,kg^{-1}$ i.v.)) without causing any major ventricular bradycardia, unlike other β-blocking drugs (Boucher & Duchêne-Marullaz, 1980). Phenoxybenzamine was used as a relatively selective α_1-antagonist, and yohimbine as a relatively selective α_2-antagonist (Minneman, 1983; Johansson, 1984; Ruffolo, 1985).

Rilmenidine and clonidine were injected 15 min after the start of atropine infusion, when the atrial rate had achieved a stable plateau, and likewise 15 min after injection of the other three antagonists. Each dose of rilmenidine and clonidine (alone or after pretreatment) was administered to a group of six dogs. A control group, composed of the same six dogs, received 0.5 $ml\,kg^{-1}$ i.v. physiological saline (0.9% W/V NaCl solution). Each injection lasted 30 s, and at least 72 h elapsed between successive tests performed on the same dog in random order. Atrial and ventricular rates, determined over 30 s, and BP were measured before and 1, 3, and 5 min after injection, and thereafter every 5 min for 1 h. To determine plasma rilmenidine concentrations in the four series where rilmenidine was given alone, blood samples were collected in three of the dogs 5, 30, and 60 min after each injection. The plasma was immediately separated by centrifuging, and frozen until assay. Plasma concentrations were determined by using a gas chromatography–negative ion chemical

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ionization mass spectrometry method (Ehrhardt, 1985; Ung *et al.*, 1987).

Drugs

Rilmenidine dihydrogen phosphate was supplied by IRI Servier (Courbevoie, France), clonidine HCl by Boehringer Ingelheim Laboratories (Reims, France), and pindolol by Sandoz Laboratories (Rueil-Malmaison, France). Atropine sulphate was purchased from Fluka (Buchs, Switzerland), pentobarbital sodium from Abbott Laboratories (Saint-Rémy-sur-Arve, France), phenoxybenzamine HCl from SKF Laboratories (Philadelphia, PA, USA), and yohimbine HCl from Sigma Chemical (St Louis, MO, USA). Doses are expressed in terms of the salt except for pindolol, for which the dose is expressed in terms of base.

Statistical analysis

Results are arithmetic means ± SEM and also mean maximal variations in rate and BP ± SEM. The latter parameter was calculated as follows: the period after which maximal or minimal mean rate or mean BP had been attained was determined during the 60 min after injection. The mean difference between corresponding individual rates or BPs and their basal values was calculated, yielding mean maximal variations ± SEM. Statistical analysis of the data was performed using analysis of variance in complete blocks without repeated measures, followed, when the *F*-value was significant, by multiple comparisons using Dunnett's test and, for comparison of the values obtained under two sets of experimental conditions, Student's *t*-test for paired samples or unpaired samples after comparison of variances. The effects of the two drugs on the same parameter were compared using either effective dose values, i.e. ED_{10}, calculated from the corresponding regression lines when possible or areas under the curves obtained by plotting the chosen parameter against time.

Results

Plasma concentrations of rilmenidine

According to the dose administered, the maximal plasma concentrations observed 5 min after injection were 2.72 ± 0.11, 5.23 ± 0.66, 12.39 ± 2.41, and 24.71 ± 3.76 ng ml^{-1}, respectively; and the minimal, 60 min after injection, 1.08 ± 0.24, 2.78 ± 0.18, 5.22 ± 0.95, and 13.16 ± 1.55 ng ml^{-1}, respectively.

Control series

Basal atrial and ventricular rates for the six dogs and mean BP for four of these dogs were 90 ± 12 and 33 ± 3 beats min^{-1} and 76 ± 7 mmHg, respectively. These values were not significantly modified after administration of physiological saline. Atrial rate remained between 87 ± 10 and 95 ± 11 beats min^{-1}, ventricular rate between 33 ± 3 and 35 ± 4 beats min^{-1}, and mean BP between 71 ± 6 and 79 ± 7 mmHg throughout the 60-min measuring period.

Effects of rilmenidine and clonidine

Atrial rate. Rilmenidine decreased atrial rate at the first minute after each injection ($P < 0.05$) (Fig. 1). This bradycardic effect, which was unrelated to dose (maximal decreases of 14 ± 5, 29 ± 6, 26 ± 7, and 28 ± 10 beats min^{-1} at 10, 25, 50, and 100 μg kg^{-1}, respectively) (Fig. 2), disappeared within 3 min of injection, and atrial rate returned to the pre-injection values and then remained stable until the end of the 60-min measuring period.

Clonidine produced a significant decrease in atrial rate at the first minute after each injection ($P < 0.05$) (Fig. 3). This effect, which was unrelated to dose (maximal decreases of 14 ± 12, 18 ± 15, 41 ± 10, and 36 ± 16 beats min^{-1} at 1, 2.5, 5, and 10 μg kg^{-1}, respectively) (Fig. 2), then disappeared, and atrial rate remained stable from the third minute until the end of the 60-min observation period.

Ventricular rate. As shown in Figs 1 and 2,

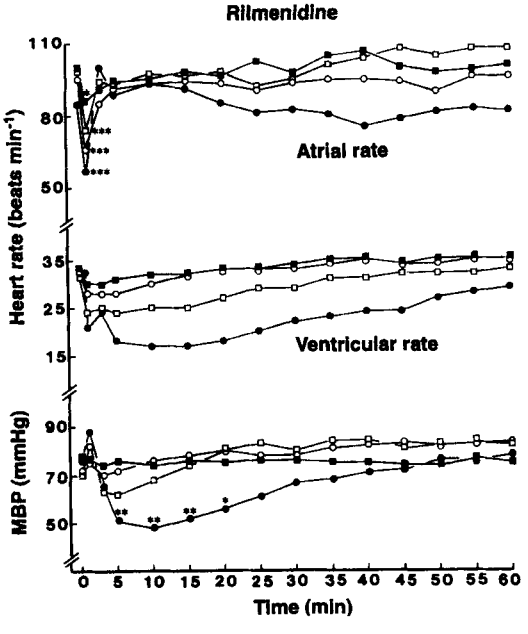

Figure 1 Changes in atrial and ventricular rates, and mean blood pressure (MBP) in conscious dogs with chronic atrioventricular block after rilmenidine: 10 μg kg^{-1} (■), 25 μg kg^{-1} (○), 50 μg kg^{-1} (□), and 100 μg kg^{-1} (●). Rilmenidine was injected intravenously at time 0. Values are means for groups of six dogs, except for MBP (only four dogs). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ in comparison with time 0 values. For clarity, all standard errors and statistical significance for ventricular rate (significant values for 3, 10, 30 and 55 min, respectively) have been omitted.

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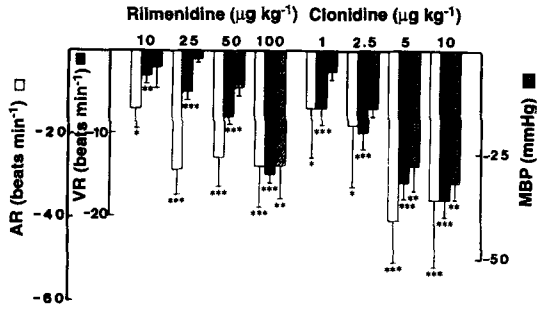

Figure 2 Maximal effects of rilmenidine ($10-100\,\mu g\,kg^{-1}$ i.v.) and clonidine ($1-10\,\mu g\,kg^{-1}$ i.v.) on atrial rate (AR) (open columns), ventricular rate (VR) (solid columns), and mean blood pressure (MBP) (hatched columns) in conscious dogs with chronic atrioventricular block. Values are means for groups of six dogs, except for MBP (only four dogs). Vertical lines show SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ in comparison with time 0 values.

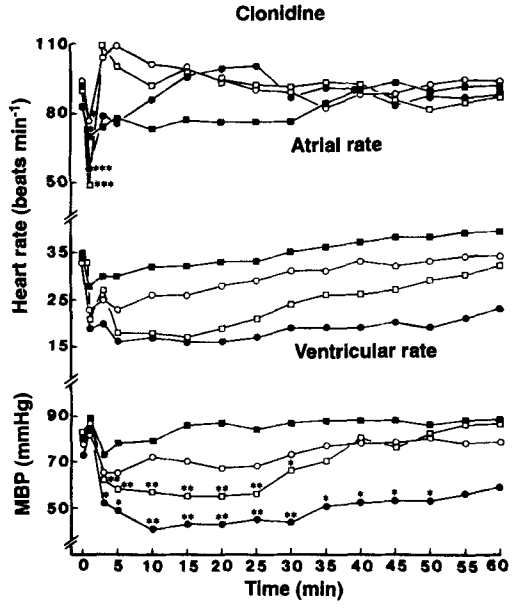

Figure 3 Changes in atrial and ventricular rates, and mean blood pressure (MBP) in conscious dogs with chronic atrioventricular block after clonidine: $1\,\mu g\,kg^{-1}$ (■), $2.5\,\mu g\,kg^{-1}$ (○), $5\,\mu g\,kg^{-1}$ (□), and $10\,\mu g\,kg^{-1}$ (●). Clonidine was injected intravenously at time 0. Values are means for groups of six dogs, except for MBP (only four dogs). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ in comparison with time 0 values. For clarity, all standard errors and statistical significance for ventricular rate (significant values for 15, 25, 45, and 60 min, respectively) have been omitted.

rilmenidine significantly decreased ventricular rate ($P < 0.01$). This bradycardic effect, which appeared at the first minute after injection, became more marked as the dose was increased, reaching 3 ± 1, 5 ± 1, 8 ± 1, and 15 ± 1 beats min^{-1} and lasting 3, 10, 30, and 55 min at 10, 25, 50, and $100\,\mu g\,kg^{-1}$, respectively. The ED_{10}, the dose producing a decrease of 10 beats min^{-1} in ventricular rate, was $52.7 \pm 2.0\,\mu g\,kg^{-1}$ rilmenidine dihydrogen phosphate, i.e. $34.1 \pm 1.3\,\mu g\,kg^{-1}$ rilmenidine base.

Clonidine produced a significant decrease in ventricular rate from the first minute onward after each injection ($P < 0.001$) (Fig. 3). This effect reached 7 ± 2, 10 ± 2, 16 ± 2, and 18 ± 2 beats min^{-1} (Fig. 2) and lasted 15, 25, 45, and > 60 min at the four doses used, respectively. The ED_{10} was $1.9 \pm 0.1\,\mu g\,kg^{-1}$ clonidine hydrochloride, i.e. $1.6 \pm 0.1\,\mu g\,kg^{-1}$ clonidine base, thus indicating that the potency ratio of rilmenidine to clonidine is about 1:21 which justifies the use of $50\,\mu g\,kg^{-1}$ rilmenidine and $2.5\,\mu g\,kg^{-1}$ clonidine after the different antagonist pretreatments.

Mean BP. Rilmenidine lowered mean BP only at the dose of $100\,\mu g\,kg^{-1}$ ($P < 0.01$) (Figs 1 and 2). This hypotensive effect reached 28 ± 8 mmHg and lasted from the fifth to the 20th minute after injection.

Clonidine at doses of $5\,\mu g\,kg^{-1}$ and above produced a decrease in mean BP ($P < 0.01$) (Figs 2 and 3). This effect, which appeared at the third minute after injection, reached 28 ± 6 and 32 ± 4 mmHg and lasted until the 30th and the 50th minute, at the 5 and $10\,\mu g\,kg^{-1}$ doses, respectively. Assessed from the respective areas under the curves, the potency ratio of the drugs was about 1:15.

Effects of the different antagonist pretreatments

The effects of the different pretreatments upon resting parameters are shown in Table 1. Blockade of muscarinic receptors with atropine and of α-adrenoceptors with yohimbine significantly increased both atrial rate ($P < 0.001$) and ventricular rate ($P < 0.05$); whereas blockade of β-adrenoceptors with pindolol only increased atrial rate ($P < 0.001$). Phenoxybenzamine did not alter atrial or ventricular rate. Mean BP was not modified by atropine, but was decreased by pindolol and phenoxybenzamine ($P < 0.01$), and increased by yohimbine ($P < 0.01$).

Table 1 Effects of the different antagonist pretreatments on resting atrial rate (AR), ventricular rate (VR) and mean blood pressure (MBP)

Drugs	AR (beats min^{-1})	VR (beats min^{-1})	MBP (mmHg)
Control	90 ± 12	33 ± 3	76 ± 7
Atropine	$185 \pm 14^{***}$	$40 \pm 5^{*}$	81 ± 6
Pindolol	$126 \pm 7^{***}$	32 ± 4	$65 \pm 7^{**}$
Phenoxybenzamine	97 ± 9	34 ± 3	$60 \pm 5^{**}$
Yohimbine	$161 \pm 18^{***}$	$51 \pm 2^{***}$	$107 \pm 8^{**}$

Values are means ± SEM for groups of six dogs, except for MBP (only four dogs).
$^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ in comparison with controls.

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Influence of muscarinic cholinoceptor blockade on the effects of rilmenidine and clonidine

Atrial rate. After muscarinic blockade with atropine, which increased atrial rate, rilmenidine ($50\,\mu g\,kg^{-1}$) decreased atrial rate from the first to the 60th minute of the observation period ($P < 0.001$), with a maximal effect at the first minute, that was not significantly different from that ovserved under basal conditions (21 ± 3 and 26 ± 7 beats min^{-1}, respectively) (Fig. 4).

After atropine pretreatment, clonidine ($2.5\,\mu g\,kg^{-1}$) produced no significant effect on atrial rate (Fig. 4).

Ventricular rate. After atropine pretreatment, which increased ventricular rate, rilmenidine decreased ventricular rate from the first to the 25th minute of the observation period ($P < 0.001$), with a maximal effect at the first minute, that was not significantly different from that produced under basal conditions (7 ± 2 and 8 ± 1 beats min^{-1}, respectively) (Fig. 4).

After atropine pretreatment, clonidine decreased ventricular rate from the first to the 25th minute of observation ($P < 0.001$), with a maximal effect at the first minute, that was not significantly different from that produced under basal conditions (10 ± 3 and 10 ± 2 beats min^{-1}, respectively) (Fig. 4).

Influence of β-adrenoceptor blockade on the effects of rilmenidine and clonidine

Atrial rate. After β-adrenoceptor blockade with pindolol, which increased atrial rate, rilmenidine decreased atrial rate from the first to the 25th minute of the observation period ($P < 0.001$), with a maximal effect at the first minute, that was not significantly different from that observed under basal conditions (31 ± 5 and 26 ± 7 beats min^{-1}, respectively) (Fig. 4).

After pindolol pretreatment, clonidine decreased atrial rate from the first to the 30th minute of observation ($P < 0.001$), with a maximal effect at the first minute that was significantly higher ($P < 0.01$) than that observed under basal conditions (39 ± 8 and 18 ± 15 beats min^{-1}, respectively) (Fig. 4).

Ventricular rate. After pindolol pretreatment, which did not affect ventricular rate, rilmenidine decreased ventricular rate only at the first minute of observation ($P < 0.05$). This decrease was significantly lower ($P < 0.01$) than that observed under basal conditions (3 ± 1 and 8 ± 1 beats min^{-1}, respectively) (Fig. 4).

After pindolol pretreatment, clonidine decreased ventricular rate only at the first minute of observation ($P < 0.05$). This decrease was significantly lower ($P < 0.01$) than that observed under basal conditions (3 ± 1 and 10 ± 2 beats min^{-1}, respectively) (Fig. 4).

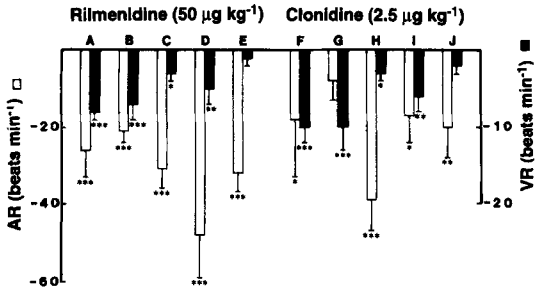

Figure 4 Maximal effects of rilmenidine alone ($50\,\mu g\,kg^{-1}$) (A), and clonidine alone ($2.5\,\mu g\,kg^{-1}$) (F), rilmenidine and clonidine after atropine (B and G), rilmenidine and clonidine after pindolol (C and H), rilmenidine and clonidine after phenoxybenzamine (D and I), and rilmenidine and clonidine after yohimbine (E and J) on atrial rate (AR) (open columns) and ventricular rate (VR) (solid columns) in conscious dogs with chronic atrioventricular block. Values are means for groups of six dogs. Vertical lines show SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ in comparison with time 0 values.

Influence of α-adrenoceptor blockade with phenoxybenzamine on the effects of rilmenidine and clonidine

Atrial rate. After α-adrenoceptor blockade with phenoxybenzamine, which did not affect atrial rate, rilmenidine decreased atrial rate from the first to the 25th minute of the observation period ($P < 0.001$), with a maximal effect at the first minute significantly higher ($P < 0.01$) than that produced under basal conditions (48 ± 11 and 26 ± 7 beats min^{-1}, respectively) (Fig. 4).

After phenoxybenzamine pretreatment, clonidine decreased atrial rate at the first minute of observation ($P < 0.05$). This decrease was not significantly different from that observed under basal conditions (17 ± 7 and 18 ± 15 beats min^{-1}, respectively) (Fig. 4).

Ventricular rate. After phenoxybenzamine pretreatment, which did not affect ventricular rate, rilmenidine decreased ventricular rate only at the first minute of observation ($P < 0.01$). This decrease was significantly lower ($P < 0.05$) than that produced under basal conditions (5 ± 2 and 8 ± 1 beats min^{-1}, respectively) (Fig. 4).

After phenoxybenzamine, clonidine decreased ventricular rate from the first to the fifth minute of observation ($P < 0.01$), with a maximal effect at the first minute significantly lower ($P < 0.05$) than that produced under basal conditions (6 ± 2 and 10 ± 2 beats min^{-1}, respectively) (Fig. 4).

Influence of α-adrenoceptor blockade with yohimbine on the effects of rilmenidine and clonidine

Atrial rate. After α-adrenoceptor blockade with yohimbine, which increased atrial rate, rilmenidine decreased atrial rate from the 35th to the 55th minute of the observation period ($P < 0.001$), with a

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maximal effect at the 45th minute not significantly different from that observed under basal conditions (32 ± 5 and 26 ± 7 beats min^{-1}, respectively) (Fig. 4).

After yohimbine pretreatment, clonidine decreased atrial rate from the 30th to the 60th minute of observation ($P < 0.01$), with a maximal effect at the 45th minute not significantly different from that observed under basal conditions (20 ± 8 and 18 ± 15 beats min^{-1}, respectively) (Fig. 4).

Ventricular rate. After yohimbine pretreatment, which increased ventricular rate, rilmenidine produced no effect on ventricular rate (Fig. 4). Under these experimental conditions, clonidine likewise produced no effect on ventricular rate (Fig. 4).

Discussion

In conscious dogs with chronic AV block, rilmenidine at i.v. doses between 10 and 100 $\mu g\,kg^{-1}$ induced an initial short-lasting (< 3 min) decrease in atrial rate, that was not related to dose. Depending on the dose and/or the route and mode of administration used, rilmenidine has been shown to either decrease or not modify heart rate (Laubie *et al.*, 1985; Dollery *et al.*, 1988; Koenig-Bérard *et al.*, 1988; Tonet *et al.*, 1991; Sannajust & Head, 1994). The atrial effect of clonidine ($1-10\,\mu g\,kg^{-1}$ i.v.) showed the same pattern. In addition, rilmenidine up to the dose of 50 $\mu g\,kg^{-1}$ and clonidine up to the dose of 2.5 $\mu g\,kg^{-1}$ did not cause mean BP to vary. At the higher doses, a hypotensive effect was always observed; rilmenidine was about 15 times less potent than clonidine. The atrial bradycardic effect is very likely of direct origin, inasmuch as the mean BP increases at the first minute (Figs 1 and 3) are very small and never significant.

Blockade of muscarinic cholinoceptors with atropine increased atrial rate only moderately compared with the effects observed in intact conscious dogs (Chassaing, Godeneche, Boucher & Duchêne-Marullaz, 1979; Rigel, Lipson & Katona, 1984), as already reported elsewhere (Boucher *et al.*, 1979; Li, Boucher & Duchêne-Marullaz, 1986; Boucher, Chassaing, Chapuy & Duchêne-Marullaz, 1994a). In addition, as expected (Boucher & Duchêne-Marullaz, 1990; Boucher, Chassaing, Chapuy & Lorente, 1994b), blockade of β-adrenoceptors with pindolol increased atrial rate. Under both experimental conditions, rilmenidine 50 $\mu g\,kg^{-1}$ produced a decrease in atrial rate, the intensity of which was identical to, and the duration longer than, that observed under basal conditions. This indicates that the initial atrial bradycardic effect of rilmenidine results from both an enhancement of cardiac cholinergic activity and a decrease in cardiac sympathetic activity, as already shown (Laubie *et al.*, 1985). This also suggests that the initial atrial bradycardia produced by rilmenidine may be buffered by a reflex tachycardic effect in response to the observed ventricular bradycardia, and/or that the value of basal heart rate may be an important factor in the development of this bradycardia. Conversely, after atropine pretreatment clonidine 2.5 $\mu g\,kg^{-1}$ produced no significant chronotropic effect on atrial rate, whereas after pindolol pretreatment, clonidine produced a decrease in atrial rate, the intensity and the duration of which were higher than those under basal conditions. This indicates that the initial atrial bradycardia observed after clonidine under basal conditions results mainly from an enhancement of cardiac cholinergic activity as suggested by literature data (Hoefke & Kobinger, 1966; Schmitt & Fénard, 1970).

Blockade of α-adrenoceptors yielded results that showed certain similarities between rilmenidine and clonidine. After phenoxybenzamine, the atrial bradycardia was either more marked than (for rilmenidine) or identical to (for clonidine) that under basal conditions; whereas after yohimbine, bradycardia was identical in intensity to basal bradycardia but with a totally different time course; the bradycardia reached its (unchanged) maximum much more slowly (45 min, compared with 1 min under basal conditions) and lasted much longer (from the 30th to the 60th minute, compared with only 1 min) (for both drugs). Though it cannot be asserted that prejunctional and/or central α_2-adrenoceptors are directly involved in the atrial bradycardic effect of rilmenidine and clonidine, as the maximal bradycardia was not reduced by yohimbine, from these results it nevertheless seems likely that these receptors are involved, at least to some extent, if only in the initiation of the effect.

Rilmenidine induced a decrease in ventricular rate, the degree of which was related to the dose administered. This effect, which was observed at plasma levels higher than 2.72 ± 0.11 ng ml^{-1}, is consistent with the absence of a negative chronotropic effect in man at plasma levels lower than 3.16 ± 0.45 ng ml^{-1} (Tonet *et al.*, 1991), and agrees with previous studies showing a slowing of heart rate after i.v. rilmenidine in animals (Laubie *et al.*, 1985; Koenig-Bérard *et al.*, 1988; Boucher *et al.*, 1994c; Sannajust & Head, 1994). Clonidine also decreased ventricular rate in a dose-related manner, in complete agreement with a previous study performed in conscious AV-blocked dogs (Duchêne- Marullaz *et al.*, 1974). Our study shows rilmenidine to be about 21 times less potent than clonidine.

As already observed (Li *et al.*, 1986; Boucher *et al.*, 1994a,b), atropine produced a slight increase in the basal ventricular rate. Under these conditions, rilmenidine and clonidine produced a decrease in ventricular rate, the intensity and duration of which were identical to those observed under basal conditions. Conversely, after pindolol, which did not modify the basal ventricular rate, in agreement with previous studies (Li *et al.*, 1986; Boucher *et al.*, 1994a,b), rilmenidine and clonidine decreased ventricular rate, with an intensity and a duration (only 1 min) of the effect that were much lower than those

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under basal conditions. These results demonstrate that the ventricular bradycardic effect of rilmenidine and clonidine results mainly from a decrease in cardiac adrenergic tone with no involvement of cardiac cholinergic activity. The slight bradycardic effect of rilmenidine and clonidine after pindolol could be due to the stimulation of negative chronotropic postsynaptic α_2-adrenoceptors, the existence of which has been shown at the ventricular level (Hordof, Rose, Danilo & Rosen, 1982; Boucher & Duchêne-Marullaz, 1990).

As in the atropine and pindolol series, the results observed after the two α-adrenoceptor antagonists were very similar for both rilmenidine and clonidine. After phenoxybenzamine, which did not affect the basal ventricular rate, rilmenidine and clonidine produced a decrease in ventricular rate, the intensity and the duration ($\leqslant 5$ min) of which were lower than those under basal conditions. This ventricular bradycardia-preventing effect can be explained by the α_2-antagonist properties reported for phenoxybenzamine (Langer & Shepperson, 1981) and/or the ability of rilmenidine and clonidine to stimulate α_1-adrenoceptors (Van Zwieten, 1988). After yohimbine, which increased the basal ventricular rate, neither rilmenidine nor clonidine produced any chronotropic effect on ventricular rate. This agrees with the study of Szabo, Urban & Starke (1993), which showed that yohimbine abolished the heart rate lowering produced by rilmenidine in conscious rabbits. It demonstrates that the ventricular bradycardia produced by rilmenidine and clonidine is mainly due to a decrease in cardiac adrenergic tone resulting from stimulation essentially of α_2-adrenoceptors, yohimbine binding only weakly with imidazoline receptors (Hamilton, Reid & Yakubu, 1988). It also confirms the previously mentioned direct effect of rilmenidine and clonidine on negative chronotropic postsynaptic α_2-adrenoceptors, as yohimbine is more potent than pindolol in preventing the ventricular bradycardia induced by rilmenidine and clonidine.

Overall, our results show that in the conscious dog rilmenidine and clonidine exert very similar bradycardic and hypotensive effects, rilmenidine being 15–20 times less potent than clonidine. Concerning the mechanism(s) of the bradycardic effect, some differences are evident between these two drugs. The rilmenidine-induced atrial bradycardia involves both a decrease in cardiac sympathetic tone and an enhancement of cardiac cholinergic activity, whereas the clonidine-induced atrial bradycardia results mainly from an enhancement of cardiac vagal activity. Both rilmenidine and clonidine induced ventricular bradycardia mainly by decreasing the cardiac sympathetic tone resulting from stimulation essentially of α_2-adrenoceptors. A direct effect of these drugs on negative chronotropic postsynaptic α_2-adrenoceptors has also to be considered in this bradycardia.

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