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CARDIOVASCULAR EFFECTS OF RILMENIDINE, A NEW α_2-ADRENOCEPTOR AGONIST, AND CLONIDINE IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS

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SUMMARY

1. The acute and chronic effects of rilmenidine, a partial agonist of α_1- and α_2-adrenoceptors with antihypertensive properties, were compared to those of clonidine on blood pressure (BP), heart rate (HR) and the urinary excretion of catecholamines, which was used as an index of sympathetic activity.

2. As these drugs are known to interfere centrally and peripherally with the sympathetic nervous system, long-term arterial blood pressure recordings in freely moving unstressed adult spontaneously hypertensive rats (SHR) were used.

3. Acute i.v. administrations of rilmenidine (0.3 mg/kg at 1200 h, 1.2 mg/kg at 1700 and 2200 h) and clonidine (12μg/kg at 1200 h, 50 μg/kg at 1700 and 2200 h) induced short-lasting increases in BP associated with a decrease in HR, which were followed by prolonged, dose-dependent decreases in BP without bradycardia. The pressor effect was less marked and the associated bradycardia was more marked in active SHR with physiologically high sympathetic activity than in resting SHR.

4. A 12-day oral treatment with rilmenidine (6.0 mg/kg daily) or clonidine (150 μg/kg daily) induced moderate decreases in BP without change in HR. Rilmenidine but not clonidine decreased normetanephrine (NMN) excretion in active but not in resting SHR.

5. Finally, during the 24 h following the cessation of the treatments, BP returned to normal, without significantly exceeding that of untreated controls. However, upswings in BP or HR were observed, more markedly and frequently after clonidine than after rilmenidine.

6. In conclusion the effects of α_2-adrenoceptor agonists appear to be influenced by the pre-existing sympathetic tone. The general agreement between these data and those observed in patients demonstrates that the use of conscious unstressed animals is of value to determine the cardiovascular effects of drugs which act on the sympathetic nervous system.

Key words: α_2-adrenoceptors, blood pressure, catecholamines, clonidine, rilmenidine, spontaneously hypertensive rat.

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INTRODUCTION

The imidazoline derivative clonidine is the prototype of antihypertensive agents which are usually considered to reduce the sympathetic tone and increase vagal tone (Kobinger 1978; Schmitt & Laubie 1983) by stimulation of α_2-adrenoceptors located in the pontomedullary region of the brain (Van Zwieten *et al.* 1983). However, most of the studies on clonidine were conducted in anaesthetized animals, in which there is an elevated sympathetic tone (Thoren & Ricksten 1979) and a decrease of baroreflex sensitivity (Fluckiger *et al.* 1985). More recently, when clonidine and related drugs were studied in conscious animals, conflicting data were obtained. For instance, in conscious spontaneously hypertensive rats (SHR) the hypotensive action was found to be associated with a bradycardia by Van Zwieten *et al.* (1986) but not by Jarrott *et al.* (1987); moreover after cessation of chronic treatment, an overshoot in heart (HR) was observed by Van Zwieten *et al.* (1986) and Atkinson *et al.* (1986) but not by Jarrott and Lewis (1987). In hypertensive subjects the plasma noradrenaline (NA) concentration, used as an index of the peripheral sympathetic activity, was reported to be decreased by Guthrie and Kotchen (1983), Struthers *et al.* (1985) and Cubeddu *et al.* (1986) but unchanged by Mohanty *et al.* (1987), while only patients with high plasma NA exhibited a decrease in the experiments of Kraft *et al.* (1987).

These discrepancies prompted us to compare the effects of clonidine and of rilmenidine, which is a new partial α_1- and α_2-adrenoceptor agonist with antihypertensive properties (Laubie *et al.* 1985; Van Zwieten *et al.* 1986), but belongs to a different chemical group than clonidine, on blood pressure (BP), HR and urinary excretion of catecholamines in conscious freely moving SHR.

METHODS

Animals

Male SHR (IFFA Credo, Les Onçins, France), 16 weeks old, were used after a 2-week habituation period to our animal house conditions (temperature 21 ± 1°C, humidity 60 ± 10% and light between 0800-2000 h). They received a standard rat chow (Entretien UAR, Villemoisson s/Orge, France) containing <0.3% sodium, with tap water *ad libitum*.

Blood pressure and heart rate measurements

Intra-arterial blood pressure (BP) and heart rate (HR) were continuously recorded in freely moving rats using our previously described computerized technique (Cerutti *et al.* 1985). A polyethylene catheter (PE 20; 0.40 mm inside diameter (i.d.)) was inserted into the lower abdominal aorta of halothane-anaesthetized rats. The catheter followed a subcutaneous route and emerged through an aluminium tube fixed to the skull of the rat. Another catheter (PE 50; 0.58 mm i.d.) was inserted into the jugular vein for intravenous (i.v.) injections. The rats were placed into metabolic cages 48 h after surgery. The aortic catheter was connected to a rotating swivel (Ets R.P., Lyon, France) and protected by a light spring attached to the tube fixed on the skull. The swivel was connected to a blood pressure transducer (Gould-Statham P23ID) by means of a three-way stopcock (Plastimed-Exaflo 8311-03) that allowed continuous infusion (0.5 mL/h) of the arterial catheter with heparin (25 units/mL) in isotonic glucose. The swivel, the perfusor and the transducer were fixed on a lever that was carefully balanced so as to allow the rats to move freely. In previous experiments, it was found that the three-way stopcock did not alter the BP curve and that, in accordance with Weeks (1971), the infusion rate used had no significant effect

on the BP level. The frequency response of the whole system (aortic catheter plus perfusion system plus transducer plus pressure processor) was evaluated using the step-function technique (Hok 1976). It was found underdamped and its resonance frequency was 36 ± 0.7 Hz. Therefore, the frequency response was satisfactory in the frequency band observed in the pressure signals of the rats (up to 30 Hz). The signal transmitted to a BP analyser (Gould Processor Amplifier 134615-52) was digitized and processed by a minicomputer Digital Minc Declab 11/23 (Digital Equipment Co.), which calculated on-line the following parameters: systolic (SBP, mmHg), diastolic (DBP, mmHg) and mean arterial pressure (MAP, mmHg) and HR (beats/min). These values were averaged every 6 s and stored on hard-discs for off-line graphical treatment and statistical analysis. Thus, we were able to study simultaneously four rats randomly allocated to the different treatments used. A 15 h habituation period (overnight) was allowed to accustom the rats to the conditions in order to obtain reliable recordings in the unstressed state.

Urinary excretion of catecholamines and methoxylated metabolites

During the collection periods, urine was shielded from light, then frozen at −80°C until assay. The contents of dopamine (DA), noradrenaline (NA), adrenaline (A) and methoxytyramine (MT), normetanephrine (NMN) and metanephrine (MN) were measured using our previously described high performance liquid chromatographic technique (Julien *et al.* 1985; Rodriguez *et al.* 1986) with both electrochemical and fluorimetric detection.

Acute i.v. administration

After a 24 h period of recording, which served as control, SHR were given either solvent (NaCl 0.9%) or rilmenidine (0.3 mg/kg at 1200 h and 1.2 mg/kg at 1700 and 2200 h) or clonidine (12 μg/kg at 1200 h and 50 μg/kg at 1700 and 2200 h). The doses were chosen to be approximately equipotent on the basis of data reported by Van Meel *et al.* (1981) in anaesthetized normotensive rats and preliminary experiments performed in our conditions.

Chronic treatment

In a preliminary study, we observed that rilmenidine given orally for 12 days at the dose of 1.2 mg/kg daily did not change the BP of conscious SHR. Therefore, in the present work, a dose of 6 mg/kg daily was selected. Groups of SHR were given rilmenidine (6 mg/kg daily) or clonidine (150 μg/kg daily) orally for 12 days (day 1 to 12) in the drinking water. The drug concentration in the water was adjusted every day on the basis of the mean water consumption observed during the two preceding days. Untreated controls were randomly assigned to each group. Surgery was performed on day 9. Starting 0800 h on day 12, BP was continuously recorded. Two urine samples were collected, one from 1200 to 2000 h (day time), and one from 2000 to 0800 h (night time). The treatment was stopped on day 13 at 0800 h and the BP monitored up to day 14 at 0800 h.

Statistical analysis

Data are expressed as mean and standard error of the mean. At any given time of the BP and HR recording, the significance of the differences between the means was assessed by the Mann-Whitney U-test. Wilcoxon's signed rank-test for paired differences, or Wilcoxon's unpaired test for comparison of two independent groups were used where appropriate.

Drugs

Rilmenidine [(N-dicyclopropylmethyl)-amino-2-oxazoline] phosphate (I.R.I. Servier, France), clonidine [2-(2,6-dichlorophenylamino)-2-imidazoline] hydrochloride (Boehringer-Ingelheim, FRG) were used. These substances were dissolved in saline and administered i.v. in a volume of 0.5 mL/kg. For the oral treatment, the substances were dissolved every day in the drinking water.

RESULTS

Single i.v. doses

Rilmenidine at the dose of 0.3 mg/kg i.v. did not significantly alter SBP nor HR, but significantly decreased DBP and MAP (Fig. 1). A dose of 1.2 mg/kg given at 1700 h (that is, in resting SHR) induced a sharp (+60 mmHg SBP) increase in BP which lasted for 15 min and was followed by a decrease (−15 mmHg SBP) which remained significant for 3.5 h. A slight, short-lasting and non-significant bradycardia was associated with the rise in BP. The same dose given at 2200 h (that is, in active SHR) induced similar effects except that the increase in BP was less marked (+40 mmHg), and the bradycardia became significant and more prolonged.

With the doses used, the BP effects of clonidine did not differ from those of rilmenidine. At a dose of 12 µg/kg, only DBP was significantly decreased without variation in HR. A dose of 50 µg/kg, given at 1700 h, induced an increase in BP (+50 mmHg SBP) lasting 30 min which was

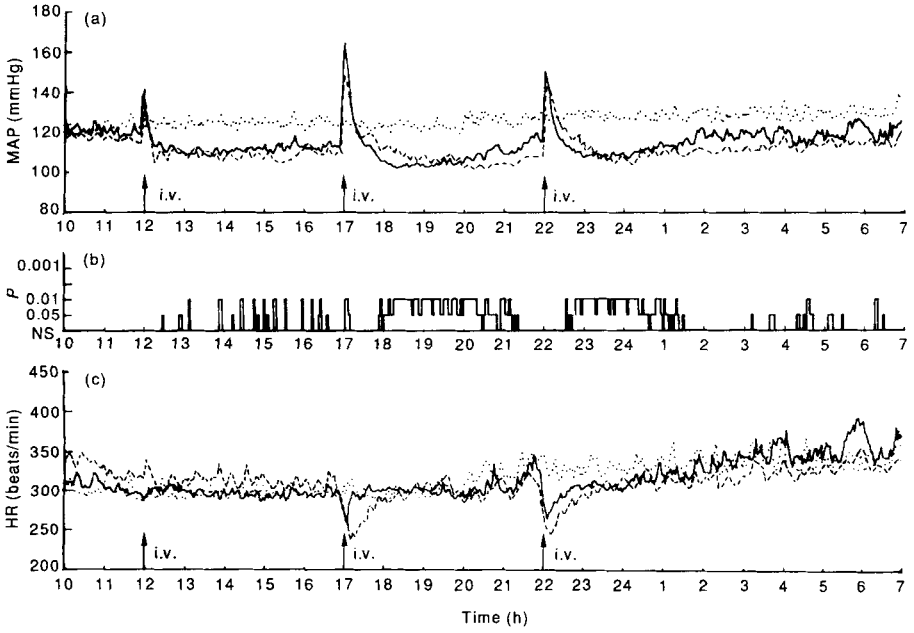


Fig. 1. Comparative effects of i.v. injections of rilmenidine (—; $n = 6$) (0.3 mg/kg at 1200 h, 1.2 mg/kg at 1700 and 2200 h) and clonidine (- - -; $n = 5$) (12 µg/kg at 1200 h, 50 µg/kg at 1700 and 2200 h) or solvent (....; $n = 6$); on (a) MAP and (c) HR of conscious SHR, during a 21 h period of recording. Each point is the mean of the values recorded during 150 s periods. Arrows show the injection times. (b) Statistical significance of the differences observed at each moment between rilmenidine-treated and control rats. There was no difference between clonidine- and rilmenidine-treated rats (Mann-Whitney U-test).

associated with a decrease in HR (−80 beats/min), and followed by a prolonged decrease in BP (−34 mmHg SBP). When given at 2200 h, the same dose induced an increase in BP which was slightly less marked, but associated with a more prolonged (1.5 h) bradycardia (−110 beats/min).

Chronic oral treatment

As indicated in Table 1, chronic oral treatment with rilmenidine (mean daily dose received = 5.96 ± 0.19 mg/kg) or clonidine (mean daily dose received = 153 ± 5 μg/kg) induced significant decreases in SBP, DBP and MAP which were stable throughout the 20 h of recording and were not associated with changes in HR.

Table 1. Mean day time and night time values of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) of conscious unrestrained SHR, at the end of a 12 day oral treatment with solvent (controls; $n = 5$) or rilmenidine (6 mg/kg daily; $n = 6$) or clonidine (150 μg/kg daily; $n = 5$) and after cessation of treatment

		Treatment (day 12)		Treatment ceased (day 13)	
		day (1200–2000 h)	night (2000–0800 h)	day (1200–2000 h)	night (2000–0800 h)
SBP (mmHg)	Controls	172 ± 3	181 ± 4	173 ± 2	182 ± 2
	Rilmenidine	162 ± 2*	166 ± 3*	162 ± 2*	168 ± 3**
	Clonidine	156 ± 7*	162 ± 3*	169 ± 4	175 ± 2*
DBP (mmHg)	Controls	111 ± 2	116 ± 2	108 ± 2	114 ± 2
	Rilmenidine	102 ± 3*	107 ± 3*	104 ± 4	109 ± 4
	Clonidine	94 ± 3*	97 ± 7*	106 ± 4	113 ± 5
MAP (mmHg)	Controls	131 ± 3	137 ± 2	130 ± 2	136 ± 2
	Rilmenidine	122 ± 3*	126 ± 3*	123 ± 3	129 ± 4
	Clonidine	115 ± 7*	119 ± 7*	127 ± 3	134 ± 3
HR (beats/min)	Controls	308 ± 8	327 ± 11	304 ± 8	335 ± 9
	Rilmenidine	287 ± 11	310 ± 10	308 ± 18	330 ± 15
	Clonidine	295 ± 5	336 ± 17	314 ± 9	355 ± 15

Statistical significance: *$P < 0.05$; **$P < 0.01$ versus controls.
Data are mean values and s.e.

Table 2 shows that, in control SHR, the urinary excretion of NA and of DA and that of their methoxylated metabolites NMN and MT increased slightly during the night as compared to the day-time. A and MN did not vary in these conditions. Rilmenidine significantly decreased the urinary excretion of NMN in active SHR, while, surprisingly, clonidine enhanced the MN excretion in resting SHR.

Wash-out period

During the 24 h following the cessation of the chronic oral administration of rilmenidine (Fig. 2), DBP, MAP and HR returned to control values while SBP remained significantly decreased for 16 h (see Table 1). After withdrawal of clonidine treatment, all the cardiovascular parameters returned to control values. It must be emphasized that, during this wash-out period, neither BP nor HR of treated rats became significantly higher than that of controls. However, wide (from 5 to 20 min) and marked upswings in BP and HR were observed in all ($n = 5$) the clonidine-treated rats and in only 2 out of the 6 rilmenidine-treated rats. In addition, these

Table 2. Effects of a 12 day oral treatment with rilmenidine (6 mg/kg daily) or clonidine (150 μg/kg daily) on urinary catecholamines and methoxylated metabolites excretion (ng/h), in conscious unrestrained SHR. Noradrenaline (NA), adrenaline (A) dopamine (DA) normetanephrine (NMN), metanephrine (MN) and methoxytyramine (MT) were measured on day 12 during day time and night time.

	NA	A	DA	NMN	MN	MT
Day time (1200–2000 h)						
Controls ($n=5$)	33 ± 5	24 ± 5	72 ± 14	51 ± 2	25 ± 1	50 ± 4
Rilmenidine ($n=6$)	31 ± 4	30 ± 7	109 ± 10	51 ± 5	31 ± 6	53 ± 3
Clonidine ($n=5$)	27 ± 8	28 ± 7	59 ± 5	52 ± 8	41 ± 6**	58 ± 3
Night time (2000–0800 h)						
Controls ($n=5$)	42 ± 11	23 ± 5	105 ± 24	69 ± 4††	24 ± 2	59 ± 6
Rilmenidine ($n=6$)	34 ± 7	28 ± 5	115 ± 30	48 ± 6**	26 ± 6	46 ± 5
Clonidine ($n=5$)	36 ± 5	32 ± 8	75 ± 16	68 ± 14	33 ± 4	52 ± 2

Statistical significance: **$P<0.01$ vs controls; ††$P<0.01$ night time vs day time.
Data are mean and s.e.; n = number of animals.

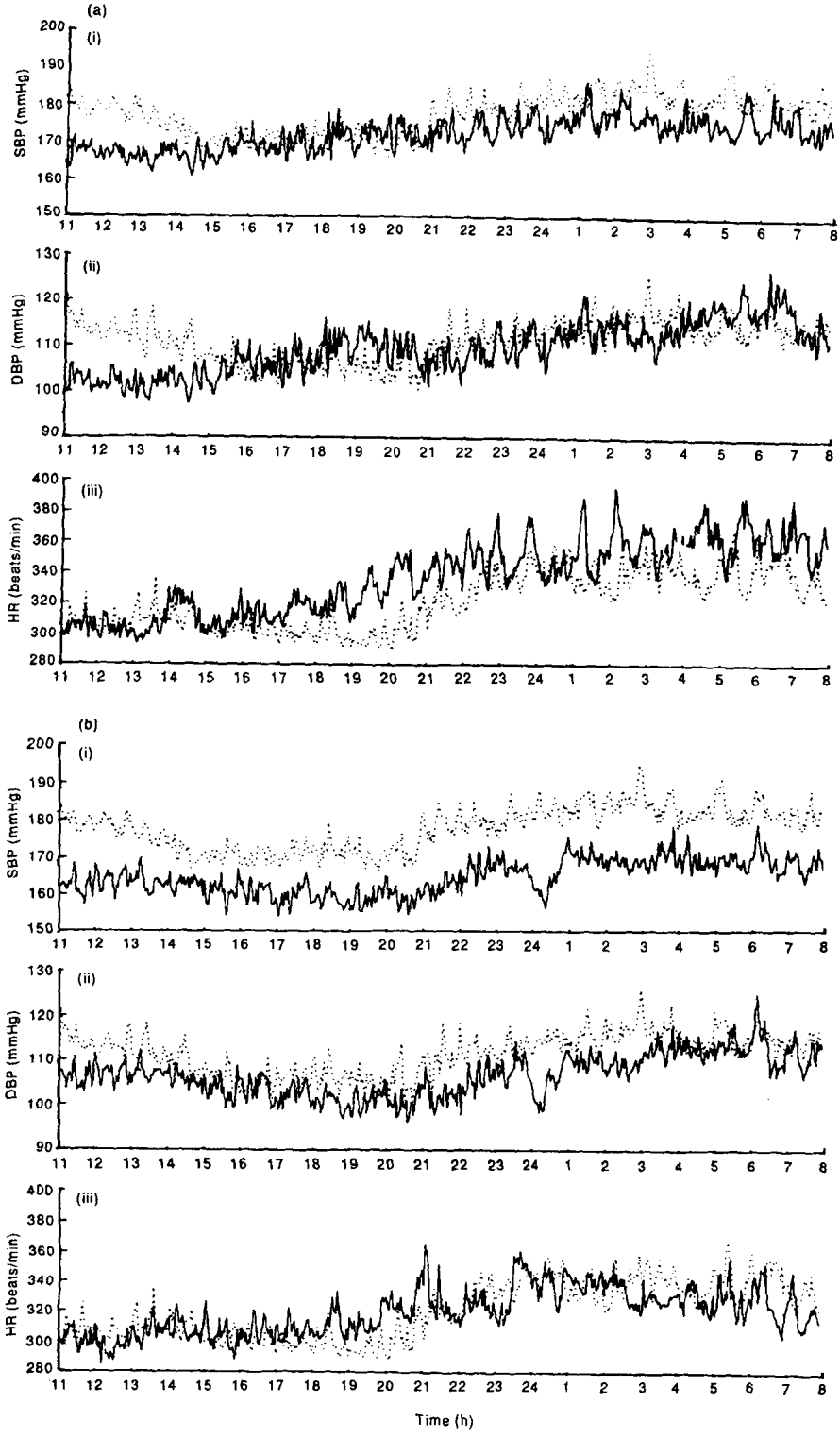
variations were less marked and frequent in rilmenidine than in clonidine-treated rats (Fig. 3).

DISCUSSION

Rilmenidine belongs to the pharmacological group of the α_2-adrenoceptor agonists with antihypertensive properties. It differs from clonidine, the reference drug of this class, by its chemical structure (oxazoline vs imidazoline), a greater selectivity for α_2- over α_1-adrenoceptors (Van Zwieten *et al.* 1986) and less sedative effects (Laubie *et al.* 1985). As this class of antihypertensive agents with α_2-adrenoceptor agonist properties is supposed to depress the sympathetic outflow by acting on the central nervous structures (Van Zwieten *et al.* 1983; Punnen *et al.* 1987) and at the periphery on the presynaptic α_2-adrenoceptors (Docherty & McGrath 1980; Gradin *et al.* 1986; Szemeredi *et al.* 1988), a precise knowledge of their cardiovascular effects requires avoidance of any interference such as anaesthesia and stress with the activity of the sympathetic nervous system. Therefore, the present experiments were devoted to the comparison of the effects of rilmenidine and clonidine on BP and peripheral sympathetic activity in conscious freely moving unstressed SHR. This was made possible by the combination of long term intra-arterial BP recordings associated with the measurement of the urinary excretion of catecholamines and methoxylated metabolites. In addition it must be emphasized that these techniques make it possible to study rats in the resting (day time: 0800–2000 h) and the active state (night time: 2000–0800 h), which renders the animal model more comparable with the situation in patients.

In these conditions, single intravenous doses of rilmenidine and clonidine induce first short-lasting, dose-dependent increases in BP due to the stimulation of adrenoceptors of vascular smooth muscle (Timmermans *et al.* 1980), associated with a bradycardia which is reflex in origin (Kobinger & Walland 1972; Laubie *et al.* 1976). These increases in BP were less marked and the associated bradycardia was more prolonged in active than in resting SHR. This suggests that the physiological increase in the sympathetic tone exhibited by active rats has two effects: first, it

Fig. 2. (i) SBP, (ii) DBP and (iii) HR evolution in conscious SHR after the cessation of a chronic oral treatment with (a) clonidine or (b) rilmenidine (each shown as solid line, $n=5$ for clonidine and $n=6$ for rilmenidine; controls are shown as dotted line, $n=5$). Each point is the mean of the values recorded during 150 s periods.


(a)
(i)
SBP (mmHg)
200
190
180
170
160
150
(ii)
DBP (mmHg)
130
120
110
100
90
(iii)
HR (beats/min)
400
380
360
340
320
300
280
11 12 13 14 15 16 17 18 19 20 21 22 23 24 1 2 3 4 5 6 7 8
(b)
(i)
SBP (mmHg)
(ii)
DBP (mmHg)
(iii)
HR (beats/min)
Time (h)

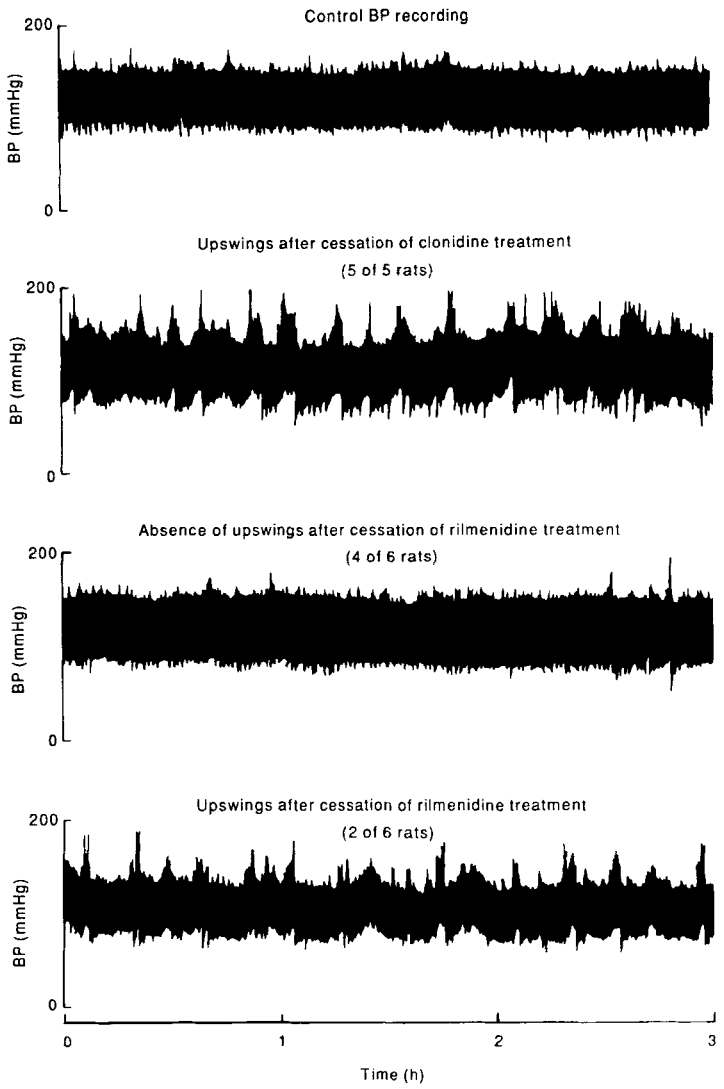


Fig. 3. BP upswings observed in conscious SHR, 8 h after the cessation of a chronic oral treatment with clonidine or rilmenidine.

down-regulates the vascular α_2-adrenoceptors and thus limits the hypertensive response, and second, it allows a more marked bradycardia to develop as the baseline HR is elevated. It must be emphasized that such increases in BP may require high plasma drug concentrations and are not of therapeutic relevance, since they were never observed in orally treated SHR. After the initial increase, DBP decreased more markedly than SBP and the decreases were dose-dependent. After the initial reflex bradycardia, HR returned to normal while BP remained decreased for 3.5 h with rilmenidine (1.2 mg/kg, i.v.) and 7 h with clonidine (50 μg/kg, i.v.). Previous studies in anaesthetized dogs (Laubie *et al.* 1985) and SHR (Van Zwieten *et al.* 1986) showed that a bradycardia was associated with the hypotensive response to single i.v. doses of rilmenidine, but they mostly took into account the initial reflex decrease in HR reported above.

After chronic oral treatment, the continuous recordings allowed us to demonstrate that, although the rats were mainly treated during the activity period, the antihypertensive effect of rilmenidine and clonidine was sustained throughout the 24 h. The decrease in BP induced by the two drugs was not associated with significant bradycardia, a result which is in accord with those recently obtained by Jarrott *et al.* (1987) in conscious SHR, but differs from those of Van Zwieten *et al.* (1986) who reported a marked bradycardia. However, in the latter study, the basal HR value (360 beats/min) of SHR was higher than in our study (300 beats/min), which suggests that the rats were partially stressed or that there were inconsistencies among the different SHR strains used.

In order to assess the extent of the sympatho-inhibition induced by the two drugs, we measured the urinary excretion of catecholamines and methoxylated metabolites, which is a reliable index of the overall peripheral sympathetic activity (Cargi & Olivero 1964). In addition, measuring urinary catecholamines also offers the interesting possibility of studying animals maintained under physiological conditions. The data obtained in control SHR showed that the activity-induced physiological increase in urinary catecholamines was less marked than in humans (Townshend & Smith 1973), a finding which is in agreement with the smaller circadian variations in BP and HR observed in rats (Su *et al.* 1986) when compared to humans. Rilmenidine treatment did not alter the excretion of A but slightly reduced that of NA and significantly that of NMN in active, but not in resting SHR. This suggests that rilmenidine is able to lower the neuronal release of NA in rats only when their spontaneous sympathetic activity is enhanced. This hypothesis is in accordance with the reduction of NA release observed by Szemeredi *et al.* (1988), using clonidine in electrically stimulated pithed rats.

In our experimental conditions, clonidine did not decrease the urinary excretion of NA or NMN. Such a result differs from other studies reporting that, in humans, clonidine decreased plasma NA (Guthrie & Kotchen 1983; Struthers *et al.* 1985; Cubeddu *et al.* 1986) and A (Chodakowska *et al.* 1987). However, Mohanty *et al.* (1987) found that clonidine did not alter basal plasma NA concentration, and Kraft *et al.* (1987) found that it reduced plasma NA only in hypertensives with pre-existing increased plasma NA concentrations. Therefore, it is possible that the discrepancy between our results and those of others could be due to the use of adult SHR which, at that age, exhibit no signs of increased sympathetic activity (Touw *et al.* 1980) when maintained in unstressed conditions. Further, it must be emphasized that the urinary excretion of catecholamines reflects the overall sympathetic tone. Since it is known that the activity of sympathetic nerves varies widely from organ to organ (Judy *et al.* 1976), our data do not exclude the possibility that rilmenidine and clonidine reduce BP by lowering the neuronal release of NA in crucial organs such as the kidneys. However our data with clonidine rule out any significant decrease in the adrenal medullary activity as suggested by Gaillard *et al.* (1987), in acutely treated normotensive rats.

Finally, when considering the 24 h after cessation of drug administration period, we found that, after clonidine withdrawal, DBP returned more rapidly than SBP to control values without overshooting them, and that the increase in HR was not significant. These findings are at variance with those of others who reported that after the cessation of clonidine treatment in normotensive rats (Thoolen *et al.* 1981a; 1982) or in SHR (Atkinson *et al.* 1986), the normalization of BP was associated with marked tachycardia. After the end of a rilmenidine treatment, SBP remained decreased while HR returned to control values. In similar conditions, Van Zwieten *et al.* (1986) observed in SHR a slow return to normal of MAP associated with a dose-dependent tachycardia while Jarrott and Lewis (1987) found no overall increase in BP and HR. Despite the lack of overshoot in BP and HR during the 24 h following the cessation of clonidine or rilmenidine treatment, some signs of withdrawal syndrome were observed, such as the short-lasting increases

in BP and HR described as upswings by Thoolen *et al.* (1981b). These increases were more frequent and more marked after clonidine than after rilmenidine treatment. Since, Jarrott *et al.* (1984) have demonstrated in normotensive rats that the intracerebroventricular injection of the α_1-adrenoceptor antagonist prazosin reduces the clonidine withdrawal syndrome, suggesting an involvement of central α_1-adrenoceptors, the differences reported here between rilmenidine and clonidine could be due to: either the higher specificity (2–3 times more) and weaker affinity (11 times less) for α_2-adrenoceptors of rilmenidine, and/or its weaker central effects.

In conclusion, in freely moving unstressed adult SHR, single intravenous doses of clonidine or rilmenidine induce, dose-dependently, short-lasting increases in BP followed by prolonged decreases of DBP more than of SBP. Chronic oral administration of both drugs produces moderate and stable antihypertensive effects without significant bradycardia. Neither rilmenidine nor clonidine decreased the urinary excretion of A, while rilmenidine lowered the excretion of NMN which provides a good index of the neuronal release of NA, in active, but not in resting rats. After cessation of chronic treatment, no overshoot of BP or HR was observed. However, BP upswings were noted, more markedly and more frequently after clonidine than after rilmenidine withdrawal. The general agreement between our findings in SHR and those made in hypertensive patients demonstrates that the continuous recording of BP and HR in unstressed rats is of value in determining the cardiovascular effects of drugs which interfere with the sympathetic nervous system, as these effects appear to be influenced by the pre-existing sympathetic activity.

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