Influence of anaesthesia on the cardiovascular effects of rilmenidine and clonidine in spontaneously hypertensive rats

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1 The acute cardiovascular effects of two α_2 -adrenoceptor agonists, rilmenidine and clonidine, were studied in 15-week-old male spontaneously hypertensive rats (SHRs). The effects of these drugs were compared with intravenous (i.v.) and intracerebroventricular (i.c.v.) administration in conscious and pentobarbitone-anaesthetized SHRs, in which aortic blood pressure (BP) was continuously recorded.

2 In conscious SHRs, i.v. doses of either rilmenidine (30, 100, 300 $\mu\text{g kg}^{-1}$) or clonidine (3, 10, 30 $\mu\text{g kg}^{-1}$) induced dose-dependent short-lasting increases in BP followed by moderate decreases associated with bradycardia, while the same three doses of both drugs given i.c.v. were devoid of BP and heart rate (HR) effects.

3 Pentobarbitone-anaesthesia increased the sympathetic control of BP and suppressed the cardiac baro-reflex sensitivity.

4 In anaesthetized SHRs, i.v. injections of the same 3 doses of rilmenidine and clonidine induced a slight increase in BP, rapidly followed by profound and long-lasting BP and HR decreases. Surprisingly, when given i.c.v., these 3 doses lowered BP and HR to the same extent but in a more progressive manner.

5 The lack of efficacy of both drugs in conscious SHRs after the i.c.v. administration of i.v. active doses and the lack of more marked and rapid effects in anaesthetized SHRs, after i.c.v. than after i.v. injections, question the involvement of a major central site of action for these antihypertensive α_2 -adrenoceptor agonists. Moreover, these results show that the cardiovascular effects of these drugs are profoundly influenced by baseline sympathetic nervous system activity which is enhanced by pentobarbitone-anaesthesia.

Keywords: α_2 -Adrenoceptors; blood pressure; conscious rats; intracerebroventricular; intravenous; pentobarbitone-anaesthetized rats; clonidine; rilmenidine; spontaneously hypertensive rats

Introduction

Rilmenidine [(N-dicyclopropylmethyl)-amino-2-oxazoline] is an oxazoline analogue of clonidine with similar anti-hypertensive properties (Laubie *et al.*, 1985; Van Zwieten *et al.*, 1986; Van Zwieten, 1988). While initially rilmenidine and clonidine were considered as potent α_2 -adrenoceptor agonists, actually, both belong to the new class of mixed α_2 -adrenoceptor/imidazoline-preferring-receptor agonists. The pharmacological profile of rilmenidine differs markedly from that of clonidine. Rilmenidine is more selective for α_2 - than for α_1 -adrenoceptors (Guicheney *et al.*, 1981; Verbeuren *et al.*, 1990) and has a 2.5 times greater selectivity for the imidazoline-preferring-receptors over cortical α_2 -adrenoceptors than clonidine (Gomez *et al.*, 1991). In addition, the main difference observed in both animals and man, is that rilmenidine induces a lower incidence of central nervous system side effects than clonidine (Laubie *et al.*, 1985; Fillastre *et al.*, 1988), lower sedative effects (Laubie *et al.*, 1985; Mahieux, 1989) and lower rebound effects during withdrawal (Sannajust *et al.*, 1989; Galley *et al.*, 1988). The mechanism of the anti-hypertensive action of rilmenidine is, like clonidine, believed to rely upon the stimulation of α_2 -adrenoceptors (Laubie *et al.*, 1985) or imidazoline-preferring-receptors (Tibirica *et al.*, 1988; Ernsberger *et al.*, 1990) in the lateralis reticularis nucleus (Bousquet & Feldman, 1987) which induces a decrease in the sympathetic nervous system (SNS) activity (Van Zwieten *et al.*, 1983; Punnen *et al.*, 1987). The assumption of such a central action is based upon several experiments showing that intracerebroventricular (i.c.v.) or intravertebral injections of clonidine (Schmitt, 1977) or of rilmenidine (Laubie *et al.*, 1985) decrease blood pressure (BP) and heart rate (HR) at doses that are ineffective by the intravenous (i.v.)

route. However, it must be emphasized that, the exact site(s) of action of these drugs within the brainstem (Van Zwieten *et al.*, 1983) as well as the receptors mediating their effects— α_2 -adrenoceptors versus imidazoline-preferring receptors—remains unclear. Furthermore, most of the above cited experiments were conducted in anaesthetized animals. Since anaesthetics usually induce sympathetic activation (Matsukawa & Ninomiya, 1989), this could have influenced the cardiovascular effects of clonidine and related drugs. Indeed, the rare studies of these drugs in conscious animals produced conflicting results. When given i.c.v., clonidine did not affect (Bonham *et al.*, 1984) or decrease BP (Kawasaki *et al.*, 1986) in normotensive conscious rats, while it did not consistently modify BP of conscious spontaneously hypertensive rats (SHRs) (Baum & Becker, 1982). In previous experiments, we observed that in conscious freely moving SHRs, rilmenidine and clonidine, administered i.v., exhibited moderate antihypertensive activity and that this effect was more marked in active rats, i.e. with an activated SNS, than in resting animals (Sannajust *et al.*, 1989). Therefore, the purpose of the present work was to evaluate the effects of anaesthesia on the cardiovascular effects of rilmenidine and clonidine given by i.v. or i.c.v. routes in adult SHRs.

Methods

Animals

Ninety 15 week-old male SHRs (IFFA-Credo, Les Oncins, France), weighing 291 to 339 g, were used. Prior to experiments, the animals were kept for a two week habituation period in our animal house under controlled conditions (temperature: $21 \pm 1^\circ\text{C}$, humidity: $60 \pm 10\%$ and lighting:

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0800 h 00 min–20 h 00 min). They received a standard rat chow (Entretien UAR, Villemoisson s/Orge, France) containing less than 0.3% sodium, with tap water *ad libitum*.

Preparation of conscious SHR_s

One week before experimentation, a stainless-steel guide-cannula (0.8 mm outer diameter, 11.0 mm length) was implanted into the right lateral cerebroventricle, under chloral hydrate anaesthesia (300 mg kg⁻¹, i.p.), according to coordinates given by De Groot (1959). Briefly, the head of the animal was placed in a small-animal stereotaxic apparatus (La Précision Cinématographique Française, Asnières, France), the incisor bar of which was positioned 3.3 mm above horizontal zero passing through the plane of the interaural line. A 1.0 mm hole was drilled perpendicular to the skull, 5.4 mm anterior and 1.7 mm lateral to the horizontal zero. The tip of the guide-cannula was positioned 7.5 mm vertically to the horizontal zero. Then, the guide-cannula was anchored to the skull with dental cement and a stylet was locked inside. After surgery, the animals received a subcutaneous (s.c.) injection of gentamycin sulphate (10 mg kg⁻¹) (Unicet Lab., Levallois-Perret, France), and were allowed to recover for 5 days before catheter implantation. This latter was performed as previously described (Cerutti *et al.*, 1985) in halothane (2% in O₂) anaesthetized animals. A polyethylene (PE) tubing composed of PE 20 (0.40 mm internal diameter (I.D.)) and PE 60 (0.76 mm I.D.) was inserted into the lower abdominal aorta and passed under the skin to emerge through an aluminum tube fixed on the skull of the animal. Another catheter (PE 50: 0.58 mm I.D.) was inserted into a jugular vein for i.v. injections. After a 2-day recovery period, the animals were placed into a cylindrical cage maintained in controlled conditions of temperature (21 ± 1°C) and lighting (08 h 00 min–20 h 00 min). The arterial line was connected to a BP transducer (Statham P 23ID, Gould Inc., Cleveland, U.S.A.) through a rotating swivel (Ets R.P., Lyon, France) which allowed the rats to move freely. An overnight habituation period was allowed before starting the recordings.

Preparation of anaesthetized SHR_s

The day of the experiment, SHR_s were anaesthetized with sodium pentobarbitone (60 mg kg⁻¹, i.p.) and two catheters composed of PE 10 (0.28 mm I.D.) and PE 50 (0.58 mm I.D.) were inserted into the left femoral artery and vein, respectively. The arterial catheter was connected to the BP transducer through a three-way stopcock to allow a continuous infusion (0.4 ml h⁻¹) of isotonic glucose containing sodium pentobarbitone (6 mg kg⁻¹ h⁻¹). Body temperature was maintained at 37 ± 1°C by means of a heating pad (Harvard Blanket Homeothermic System, Millis, U.S.A.). The trachea was cannulated for artificial ventilation (Harvard apparatus, model 683, Millis, U.S.A.) with room air (10 ml kg⁻¹ × 65 min⁻¹). The animal was then placed in the stereotaxic apparatus and a guide-cannula was implanted into the right lateral cerebroventricle as previously described. At the beginning and at the end of each experiment, arterial blood gases and pH were measured with a blood gas and pH analyser (Instrumentation Laboratory, model 213, Milano, Italy).

Data collection

Arterial BP and HR were continuously recorded in freely moving and pentobarbitone-anaesthetized animals by a computerized technique (Gustin *et al.*, 1990). Briefly, the BP signal was transmitted to a BP analyser (Gould Processor Amplifier 134615-52, Cleveland, U.S.A.), then digitized and processed by a computer (SYS-3304/NY/082, Motorola Microsystems, Tempe, U.S.A.) which allows real-time, beat-by-beat determination of systolic (SBP) and diastolic (DBP) BP, mean arterial pressure (MAP) and HR, graphical monitoring and data

storage on cartridge tapes (DC 600A, 3M, Germany). Off-line subsequent data analysis such as graphical and statistical analysis was performed on a workstation (SUN 3/50M, Sun Microsystems, Mountain View, U.S.A.).

Sympathetic control of arterial pressure

Sympathetic control was assessed at the beginning of each experiment as the maximum BP decrease induced by the short acting ganglion blocker, trimethaphan camsilate (10 mg kg⁻¹, i.v.). Previous experiments, using direct measurement of the renal sympathetic nerve activity in conscious and anaesthetized SHR_s, have demonstrated that this dose of trimethaphan totally abolishes, for a few minutes, the electrical activity of the postganglionic renal fibers (unpublished observation).

Cardiac baroreflex sensitivity

Cardiac baroreflex sensitivity was determined by the beat-by-beat study of the changes in SBP and heart period (HP) [60 000/HR: ms] induced by i.v. injections of phenylephrine (Phen, 5 µg kg⁻¹) and nitroglycerin (NG, 100 µg kg⁻¹) (Smyth *et al.*, 1969). As previously described (Su *et al.*, 1986), for each injection the computer calculated the delay between the drug-induced SBP and HP changes that gave the best linear relationship between these two parameters. When the correlation coefficient reached the $P < 0.001$ significance level, the slope (ms mmHg⁻¹) of this relationship was used as an index of the baroreflex sensitivity. For each rat, the baroreflex sensitivity was expressed as the mean of the slopes obtained after three injections of Phen and NG.

Assessment of the guide-cannula implantation

Two criteria were used to assess guide-cannula implantation. Firstly, it was checked that i.c.v. injections of angiotensin II (300 ng kg⁻¹) induced both a drinking behaviour, within 1–2 min, and a pressor response in conscious animals (Hoffman *et al.*, 1977) and a pressor response in anaesthetized animals. Secondly, at the end of each experiment 2 µl of methylene blue dye [5% in artificial cerebrospinal fluid (CSF)] was injected and the brain was removed for histological examination. Correct implantation was assumed when heavy staining of the ventricles, including the fourth ventricle, could be observed.

Experimental protocols

In all the experiments, the injected volumes were 0.5 ml kg⁻¹, i.v. and 2 µl, i.c.v. per animal. The ascertainment of the stability of the preparations during the course of the experiments was previously described (Sannajust *et al.*, 1989); therefore two series of experiments were designed.

Conscious SHR_s BP and HR were recorded for 0.5 to 1 h (control period). Then each animal successively received one i.v. injection of trimethaphan followed, 1 h later by three i.v. bolus injections of Phen and NG. When all the cardiovascular parameters had returned to control values, SHR_s were randomly allocated to i.v. or i.c.v. injections and to rilmenidine (i.v.: $n = 7$; i.c.v.: $n = 6$) or clonidine (i.v.: $n = 6$; i.c.v.: $n = 6$) groups. Each rat received three successive increasing doses of rilmenidine (30–100–300 µg kg⁻¹) or of clonidine (3–10–30 µg kg⁻¹) given at 3 h intervals.

Pentobarbitone-anaesthetized SHR_s After an equilibration period which allowed the cardiovascular parameters, blood gases and pH to stabilize, BP and HR were recorded for a 0.5 h control period. Then each animal received trimethaphan and 1 h later, 3 injections of Phen and of NG. When all the parameters had returned to control values the rat received, either i.v. or i.c.v., one dose of rilmenidine (30 or 100 or 300 µg kg⁻¹) or clonidine (3 or 10 or 30 µg kg⁻¹), which was preceded by the injection of corresponding vehicle. Therefore, 12

groups of rats (all $n = 5-6$) were studied and the route of administration, drug and dose were randomized.

Statistical evaluation

Data are expressed as mean $\pm$ s.e.mean. Wilcoxon's signed rank-test for paired differences or Wilcoxon's unpaired test for comparison of two independent groups were used when appropriate.

Drugs and solutions

Rilmenidine [N-(dicyclopropylmethyl)-amino-2 Δ 2-oxazoline] phosphate (I.R.I. Servier, France), clonidine [2-(2,6-dichlorophenyl)-amino-2 imidazoline] hydrochloride (Boehringer-Ingelheim, Germany) were dissolved either in artificial CSF and maintained in sealed vials at 37°C or in sterile saline, for i.c.v. or i.v. administrations respectively. The artificial CSF (Palaic *et al.*, 1967) contained (millimolar): NaCl 126.5, KCl 2.4, CaCl₂ 1.30, MgCl₂ 0.83, NaHCO₃ 27.5, KH₂PO₄ 0.50, urea 2.7 and glucose 3.9. It was stored at -4°C, pre-heated to 37°C and adjusted to pH 7.3 immediately before starting the i.c.v. injections. [Val⁵]-angiotensin II acetate was obtained from Sigma Chemical Company (St Louis, U.S.A.). Trimethaphan camsilate (Hoffmann-La-Roche Lab., Basel, Switzerland), phenylephrine chlorhydrate (Sigma Lab., St Louis, U.S.A.) and nitroglycerin (Besins-Iscovesco Lab., Paris, France) were dissolved in sterile saline.

Results

Studies in conscious SHR

As presented in Figure 1, i.v. injections of three doses of rilmenidine induced a sharp dose-dependent BP increase which lasted for 5 to 10 min and was followed by a progressive and long-lasting dose-dependent decrease. The maximal effect reached: 18 ± 3 , -26 ± 3 and -34 ± 2 mmHg for SBP and -13 ± 3 , -16 ± 4 and -20 ± 3 mmHg for DBP at the doses of 30, 100 and $300 \mu\text{g kg}^{-1}$, respectively. These BP changes were significant at the $P < 0.05$ level for the two lower doses and at the $P < 0.01$ level for the $300 \mu\text{g kg}^{-1}$ dose and were associated with a slight bradycardia which did not reach the level of statistical significance (Figure 3). Clonidine (Figure 2) induced similar decreases in BP (SBP: -20 ± 2 , -28 ± 3 and -34 ± 4 mmHg; DBP: -11 ± 1 , -18 ± 3 and -22 ± 4 mmHg for the doses of 3, 10 and $30 \mu\text{g kg}^{-1}$, respectively). These decreases were statistically significant at the $P < 0.01$ level for SBP and DBP only for the $30 \mu\text{g kg}^{-1}$ dose and at the $P < 0.05$ level for DBP at the lower doses. Therefore, in conscious SHR, the equipotent rilmenidine/clonidine ratio is approximately 10. However, the effects of clonidine differed from those of rilmenidine in that the hypertensive peak was more marked and there was a more marked and long-lasting bradycardia which reached statistical significance (-130 ± 5 b.p.m., $P < 0.05$) only for the $30 \mu\text{g kg}^{-1}$ dose. As indicated in Figure 3, clonidine $30 \mu\text{g kg}^{-1}$, i.v.

Figure 1 Changes in systolic (SBP), diastolic (DBP) blood pressure and heart rate (HR) before and after i.v. (a, b) or i.c.v. injection (c, d) of rilmenidine in one conscious (a, c) and one anaesthetized (b, d) 15 week-old male spontaneously hypertensive rat. Each point of the graph is the mean of the values recorded during an 80 s period.

Figure 2 Changes in systolic (SBP), diastolic (DBP) blood pressure and heart rate (HR) before and after i.v. (a, b) or i.c.v. injection (c, d) of clonidine in, one conscious (a, c) and one anaesthetized (b, d) 15 week-old male spontaneously hypertensive rat. Each point of the graph is the mean of the values recorded during an 80 s period.

decreased HR to the level observed after ganglionic blockade with trimethaphan.

I.c.v. injections of angiotensin II (300 ng kg^{-1}) induced an almost immediate drinking behaviour which lasted for 10 to

15 min and was associated with a SBP increase of $28 \pm 3 \text{ mmHg}$ (Table 1). As indicated in Figures 1, 2 and 3, neither rilmenidine nor clonidine when given i.c.v., induced significant changes in BP and HR.

Figure 3 Maximum decreases in mean arterial pressure (ΔMAP) and heart rate (ΔHR) after administration of rilmenidine (a, b) by i.v. (□) or i.c.v. (■) routes, or of clonidine (c, d) by i.v. (○) or i.c.v. (●) routes in groups of 6 conscious or pentobarbitone-anaesthetized 15 week-old male spontaneously hypertensive rats. The hatched area corresponds to the mean ($\pm$ s.e.mean) values observed after an i.v. injection of trimethaphan (10 mg kg^{-1}).

* $P < 0.05$ vs i.c.v.

Table 1 Effects of pentobarbitone-anaesthesia on the cardiovascular parameters of 15 week-old male SHR

	Conscious (n = 13)	Pentobarbitone (n = 65)
<i>Baseline</i>		
SBP (mmHg)	198 ± 3	193 ± 2
DBP (mmHg)	120 ± 3	147 ± 2**
MAP (mmHg)	146 ± 3	163 ± 2**
HR (b.p.m.)	324 ± 14	364 ± 3**
<i>Effects of trimethaphan (10 mg kg⁻¹, i.v.)</i>		
ΔSBP (mmHg)	-70 ± 5	-105 ± 3**
ΔDBP (mmHg)	-58 ± 4	-101 ± 3**
ΔMAP (mmHg)	-62 ± 3	-103 ± 3**
ΔHR (b.p.m.)	-78 ± 10	-97 ± 6
<i>Cardiac baroreflex sensitivity (ms mmHg⁻¹)</i>		
Phen	0.62 ± 0.11	0.13 ± 0.02**
NG	0.86 ± 0.11	0.06 ± 0.01**
<i>Angiotensin II (300 ng kg⁻¹, i.c.v.)</i>		
ΔSBP (mmHg)	28 ± 3	15 ± 1**
ΔDBP (mmHg)	26 ± 2	11 ± 1**
ΔMAP (mmHg)	27 ± 2	12 ± 1**
ΔHR (b.p.m.)	16 ± 4	6 ± 2*

Values are means ± s.e.mean. For abbreviations see text.

* $P < 0.05$; ** $P < 0.01$ vs conscious.

Effects of pentobarbitone-anaesthesia

As indicated in Table 1, pentobarbitone slightly increased baseline HR and significantly enhanced DBP. The influence of SNS on BP was markedly increased by pentobarbitone anaesthesia as demonstrated by the greater hypotensive response to the ganglionic blocker, trimethaphan, and the cardiac baroreflex sensitivity was almost abolished, when measured after Phen and NG injections. Finally, the pressor effects of i.c.v. angiotensin II were lower in anaesthetized animals than in conscious rats. Blood gases and pH were stable throughout the experiment and were as follows: before PO_2 : 86 ± 0.2 mmHg, PCO_2 : 35 ± 0.6 mmHg and pH: 7.47 ± 0.007 , and at the end of the experiments, PO_2 : 84 ± 0.7 mmHg, PCO_2 : 37 ± 0.7 mmHg and pH: 7.47 ± 0.009 .

Studies in pentobarbitone-anaesthetized SHR

The effects of i.v. injection of increasing doses of rilmenidine and clonidine in pentobarbitone-anaesthetized SHR were strikingly different from those observed in conscious conditions. As shown by Figures 1 and 2, the immediate pressor effect was almost abolished. Most significantly, both rilmenidine and clonidine evoked a rapid and profound decrease in BP and in HR. Both parameters tended to return to baseline more rapidly after rilmenidine than after clonidine, but remained significantly lowered for the 4 h of observation. Figure 3 indicates that the peak effects of rilmenidine were dose-dependent, while the lowest dose used of clonidine ($3 \mu\text{g kg}^{-1}$) evoked the maximum antihypertensive ($P < 0.001$) and bradycardic ($P < 0.001$) effects. Therefore, in anaesthetized SHR, the equipotent rilmenidine/clonidine ratio was at least 100.

When considering the i.c.v. injections, both drugs (Figures 1 and 2) evoked a profound and long-lasting decrease in BP and HR, the magnitude of which was similar to that observed after the i.v. injection of the same doses (Figure 3). However, the effects of i.c.v. injections differed from those of i.v. administration by the absence of a short lasting preliminary pressor response and by a more progressive decrease in BP. Finally, the maximum BP and HR effects of rilmenidine and clonidine did not differ from those of trimethaphan, except that the maximum bradycardic response obtained after i.c.v. adminis-

tration of the lowest dose of clonidine was greater than that of trimethaphan.

Discussion

The mechanisms whereby rilmenidine (an original anti-hypertensive compound with an oxazoline ring) and clonidine (the prototype of central antihypertensive agents with an imidazoline structure) lower BP and HR have been related to a decrease in the peripheral SNS activity mediated by the stimulation of postsynaptic α_2 -adrenoceptors located within the central nervous system (for review see Schmitt, 1977; Van Zwieten *et al.*, 1983). However, the precise site(s) of action of clonidine remains unclear (Jarrott *et al.*, 1987) and it has recently been suggested that the receptors involved differ from α_2 -adrenoceptors and are more specific for the imidazole structure (Meeley *et al.*, 1986; Lachaud *et al.*, 1988; Ernsberger *et al.*, 1990). Furthermore, in previous experiments (Sannajust *et al.*, 1989), we observed that in conscious SHR, rilmenidine like clonidine induced significant but mild decreases in BP and HR, associated with a slight reduction in the urinary excretion of catecholamines taken as an index of the peripheral SNS activity. The discrepancy between these results and most of those of the literature prompted us to assess the importance of a central site of action and the effects of anaesthesia on the cardiovascular effects of rilmenidine and clonidine.

For that purpose, drugs were given by the i.v. and i.c.v. routes. In conscious freely moving SHR, the results obtained after i.v. injections of rilmenidine or clonidine suggest that, besides a reduction in the peripheral SNS activity, clonidine more than rilmenidine lowers HR by another mechanism, which has been proposed to be a central activation of the vagal tone (Schmitt & Laubie, 1983). However, the most striking result was that the doses of rilmenidine and clonidine, which were active when given i.v., were devoid of cardiovascular effects when administered i.c.v.

As we have postulated previously (Sannajust *et al.*, 1989) that the importance of the effects of rilmenidine were related to the level of the sympathetic drive of the animals. The experiments were repeated in rats anaesthetized with pentobarbitone, an agent that enhanced the SNS tone (Touw *et al.*, 1980) and almost abolished the cardiac baroreflex sensitivity. Under these conditions, when given i.v., we observed consistent differences in the BP effects of the two drugs. The absence of initial pressor effect of i.c.v. injections of rilmenidine and clonidine in anaesthetized SHR is likely to be due to the lack of stimulation of α_1/α_2 -vascular adrenoceptors (Kobinger & Pichler, 1975; Timmermans & Van Zwieten, 1980) by drugs leaking from the cerebral ventricle to the systemic circulation. Moreover, the slower onset of antihypertensive action after i.c.v. than after i.v. administration, may represent the time needed for the small i.c.v. injected volume ($2 \mu\text{l}$) to reach peripheral sites of action, such as those in the medulla oblongata (Sun & Guyenet, 1986; Punnen *et al.*, 1987) or the thoracolumbar spinal cord (Connor & Finch, 1981; Solomon *et al.*, 1989). This finding, which is especially clear with rilmenidine, argues against the hypothesis of a preferential central action, as it is difficult to conceive that the same amount of drug would more rapidly reach the medulla oblongata when given i.v. than when given i.c.v. Several studies have examined the disposition of clonidine in rats after i.v. administration. Conway & Jarrott (1980) found that the administration of clonidine ($20 \mu\text{g kg}^{-1}$, i.v.) led to a rapid distribution, with the highest levels being obtained in all the tissues 2 min after the injection. At the peak of the antihypertensive response, less than 2% of the injected dose was present in the brain. These results emphasize that a considerable amount of clonidine does not enter the central nervous system and that a peripheral action of the antihypertensive effect of clonidine must be considered. Therefore as: (1) in anaesthetized SHR i.v. doses were found as potent and more rapidly active than i.c.v. doses

and, (2) in conscious SHRs the doses which were active i.v. were devoid of effect when given i.c.v., it may be proposed that both drugs do not act primarily on the central nervous system to lower BP and HR and that they perhaps do not work through identical mechanisms. Moreover, the lack of effect of i.c.v. rilmenidine or clonidine in conscious SHRs raises several hypotheses. The use of insufficient doses or the injection of a volume too small to reach the ventral part of the medulla oblongata can be ruled out on the basis of the efficacy of the same doses i.v. in conscious SHRs and of the same doses given i.c.v. in anaesthetized animals. Another possibility is that clonidine and related drugs exert different cardiovascular effects by acting on different nuclei of the brain. Kawasaki & Takasaki (1986) observed that i.c.v. BHT-920, another α_2 -adrenoceptor agonist, exhibited pressor effects in conscious rats while it was depressor in pentobarbitone-anaesthetized animals. They speculated that BHT-920 could be pressor by acting on suprabulbar structures and depressor on bulbar sites, and that anaesthesia had specifically suppressed the suprabulbar action. Such an hypothesis which suggests that the pressor and depressor effects of rilmenidine or clonidine act in balanced opposition in conscious SHRs, has been recently ruled out by Gomez *et al.* (1991), who demonstrated that decerebration did not change the cardiovascular effects of rilmenidine.

Therefore, the most likely explanation for the marked differences observed between the effects of i.c.v. rilmenidine and clonidine in conscious and anaesthetized animals may involve

the pentobarbitone-induced changes in SNS activity and in the cardiac baroreflex sensitivity. In anaesthetized SHRs with an elevated sympathetic drive, the sympathoinhibitory action of rilmenidine and clonidine can be fully expressed. In addition, it is not opposed by a baroreflex activation. In freely moving and unstressed SHRs, with a low SNS activity, clonidine or rilmenidine can exert only small sympathoinhibitory effect. This slight effect is opposed by the baroreflex activation; thus Bonham *et al.* (1984) demonstrated that, when given i.c.v. clonidine was devoid of effects in conscious normotensive rats but became hypotensive after sinoaortic denervation.

In conclusion, this study does not allow the exclusion of a central action of rilmenidine and clonidine. However, it clearly demonstrates that, if such an action occurs, it may not be of major importance in the cardiovascular effects of these drugs when they are given in conscious and unstressed SHRs with a normal baroreflex sensitivity. In addition, it shows that the ability of these two drugs to lower BP could be related to the level of sympathetic drive. Finally, the experiments described in the paper show the importance of conscious animals for the study of antihypertensive agents which interfere with the autonomic nervous system.

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