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Rilmenidine and reflex renal sympathetic nerve activation in wistar and hypertensive rats

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1 This study sets out to examine the effect of rilmenidine administered systemically on basal and reflexly activated renal nerve activity in Wistar and stroke prone spontaneously hypertensive rats (SHRSP).

2 Animals were anaesthetized with chloralose/urethane, stimulating electrodes were placed on the brachial plexi and the renal nerves were isolated and put on recording electrodes. Both brachial nerves were stimulated electrically at 0.8, 1.6 and 3.2 Hz (15 V, 0.2 ms) in the absence and in the presence of rilmenidine given at 100 and 200 $\mu g\ kg^{-1}$ i.v. in a cumulative manner.

3 Stimulation of the brachial nerves caused graded increases in blood pressure, heart rate and integrated renal nerve activity ($P<0.05$) in both Wistar and SHRSP. Fast Fourier transformation of the renal nerve activity signal to generate a power spectrum demonstrated that both total power and percentage power at heart rate was higher in the SHRSP than Wistar ($P<0.05$). Total power was raised during brachial nerve stimulation in both Wistar and SHRSP by some 200-300% ($P<0.05$) but the percentage power at heart rate was decreased by some 60% ($P<0.01$) in the Wistar but was raised by some 40-50% ($P<0.05$) in the SHRSP.

4 Administration of rilmenidine caused dose-related decreases in blood pressure and heart rate and integrated renal nerve activity in both Wistar and SHRSP (all $P<0.05$). Both doses of rilmenidine decreased ($P<0.05$) the total power in the signal in both strains of rat by about one-half but the power occurring at heart rate only fell at the higher dose of compound in the Wistar, whereas in the SHRSP it was decreased by both doses by approximately 60-70%. In the presence of rilmenidine, coherence of the renal nerve signal was reduced in the Wistar and SHRSP and although the drug had no effect on phase difference in the Wistar, this parameter was decreased in the SHRSP by the low and high doses of rilmenidine ($P<0.05$).

5 In the presence of 100 $\mu g\ kg^{-1}$ rilmenidine, stimulation of the brachial nerves caused increases in total power in the Wistar and SHRSP (two to three fold, $P<0.05$), together with a decrease ($P<0.05$) in the percentage power occuring at heart rate in the Wistar, of some 60%, and an increase ($P<0.01$) in the SHRSP, of some two to three times, which were very similar in magnitude and pattern to those obtained in the absence of the drug. Following the 200 $\mu g\ kg^{-1}$ dose of rilmenidine, brachial nerve stimulation increased total power in the Wistar and SHRSP groups ($P<0.05$) and whereas in the Wistar the percentage power at heart rate did not change in the SHRSP it was again increased in response to the electrical stimulation of the brachial plexus ($P<0.001$) by between two to three fold.

6 These results showed that in both the Wistar and SHRSP rilmenidine depressed blood pressure, heart rate and integrated renal nerve activity. Moreover, rilmenidine did not affect the reflex activation of renal nerve activity via the somatosensory system although the characteristics within the power spectra underwent certain changes which might have a functional impact at the level to the kidney.

Keywords: Renal nerve activity; rilmenidine; imidazoline receptor agonists; hypertension

Introduction

It is increasingly recognised that the neural regulation of the kidney, in terms of haemodynamics, tubular function and renin release, may play a major role not only in the genesis of hypertension (Wyss *et al.*, 1992) but also in the maintenance of the chronically elevated blood pressure (Panfilov & Reid, 1994). Furthermore, there is evidence that the renal sympathetic nerves have an exaggerated influence on kidney function in experimental models of myocaridal infarcts (DiBona & Sawin, 1994) and ascites (DiBona & Sawin, 1991). The cause of the excessive neural influences on the kidney under these circumstances is unclear, but may result from deficits in the high pressure carotid sinus and aortic arch baroreceptors or on the low pressure cardiopulmonary baroreceptors which have a major impact on renal sympathetic outflow. Alternatively, there may be an inappropriate sympathetic outflow due to central nervous system derrangements, or inappropriate interactions with other systems as the nerve traffic progresses from the central nuclei to the periphery.

It has been recognised for some time that α_2-adrenoceptor agonists, such as clonidine, have an action at the central nervous system in the region of the medulla to decrease sympathetic outflow. However, structure-activity relationships demonstrated that the hypotensive action was more related to the imidazoline structure of the compound rather than its α_2-adrenoceptor activating action (Bousquet, 1995). From these studies, the concept grew that there is a class of imidazoline receptors which are activated by endogenous ligands, the nature of which has yet to be established, and have given rise to a number of synthetic compounds with antihypertensive actions, the first generation of which is rilmenidine (Dubar & Pillion, 1995).

There is evidence showing that rilmenidine acts at imidazoline receptors, which are present within the rostral venterolateral medulla (Ernzberger *et al.*, 1990), to cause a decrease in sympathethic outflow with a subsequent decrease in total peripheral resistance and heart rate which contributes to the fall in blood pressure. A number of studies in the rat (Sannajust *et al.*, 1992; Kline & Cechetto, 1993) and rabbit (Szabo *et al.*, 1993; Head *et al.*, 1993) showed that both central and peripheral administration of rilmenidine causes an inhibition

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of sympathetic nerve activity to the kidney. This renal sympatho-inhibition was more apparent in spontaneously hypertensive rats (SHR) and the magnitude of the inhibition was dose-dependent. Furthermore, it has been shown (Sannajust & Head, 1993) in the conscious rabbit that rilmenidine dose-dependently depresses the baroreflex control of renal sympathetic nerve activity.

At the level of the kidney, the renal nerves cause renin release, stimulate tubular sodium reabsorption and, at high levels of activation, cause reductions in renal haemodynamics (Osborn & John, 1989). Thus, not only does removal of renal nerve effects by surgical section cause an increase in both sodium and water excretion, but also activation of the nerves, either by direct electrical stimulation (Johns & Manitius, 1987) or via physiological reflexes (Davis & Johns, 1994a, b) cause an antinatriuresis and antidiuresis. Rilmenidine administration has been shown to cause a natriuresis and diuresis; part of this effect is dependent on the renal nerves (Kline & Cechetto, 1993) and probably represents a withdrawal of renal sympathetic tone. However, it is clear that within the kidney itself, rilmenidine interferes with tubular sodium reabsorption (Li *et al.*, 1994), probably at the proximal tubule (Rouse *et al.*, 1990; Gargalides-Moudanos & Parini, 1995). It is likely that these indirect and direct actions of rilmenidine could enhance the mobilization of fluid, particularly in the hypertensive state which would prove most beneficial in normalising blood pressure.

What has yet to be assessed is whether administration of these imidazoline type compounds could suppress not only the basal outflow of renal sympathetic activity but also its reflex activation. This was addressed in the present study by investigating the activation of the somatosensory system by electrical stimulation of the brachial plexus, which we have previously shown to increase renal sympathetic nerve activity and to cause a renal nerve-dependent antinatriuresis and antidiuresis (Davis & Johns, 1994b). In order to gain further insight, renal nerve activity was subjected to fast Fourier transformation to generate a power spectrum which gives the proportion of energy within the signal which occurs at each frequency over a chosen range (Davis & Johns, 1995). This has advantages over conventional multifibre recordings of integrated nerve activity which suffer from being highly dependent on the number of fibres present in the nerve and damage which takes place during dissection. A number of parameters can be derived from the power spectrum, the percentage of power at heart rate, which indirectly reflects the baroreceptor reflex, coherence, which measures the correlation between the blood pressure and renal nerve signal, and phase difference which estimates the relationship between the two signals. All of these parameters are the result of central nervous system integration and any changes will be a consequence of altered brain function.

Methods

Male Wistar and stroke-prone spontaneously hypertensive (SHRSP) rats, 280–330 g were fasted, but given access to water, during the night before use. Anaesthesia was induced with a mixture of fluothane/N_2O/O_2, a femoral vein cannulated, a continuous intravenous infusion of saline (150 mmol NaCl) initiated at 3 ml h^{-1} and the anaesthetic was gradually (over 30 min) replaced with a urethane/chloralose mixture, i.v. (180 mg and 12 mg, respectively). A femoral artery was cannulated for monitoring blood pressure (CEC Instrumentation pressure transducer and amplifier, Grayden Electronics, Birmingham). The animals were tracheotomised and breathed spontaneously. Both brachial nerve plexi were isolated, placed on bipolar stimulating electrodes insulated from the surrounding tissues with parafilm and held in place with dental impression cement (Impregnum F, Seefeld, Germany). The bladder was cannulated to allow urine to drain, the left kidney exposed via a retroperitoneal incision and its ureter cannulated. The renal nerves were carefully dissected, cleaned and placed on bipolar stainless steel (Medwire, New York, U.S.A.) electrodes and once a pulsatile signal could be observed, they were sealed in place with Wacker sil gel 604 (Wacker, Munich, Germany). The animals were allowed 2 h to recover from the surgery.

Protocol

A 3.5 min collection of blood pressure and renal nerve activity was undertaken to record baseline levels and then the brachial nerves were stimulated bilaterally at (15 V, 0.2 ms) 0.8 Hz and once all parameters had stabilised (within 15–30 s), a further 3.5 min collection period was carried out. The stimulation was stopped and following a 5 min recovery period, a further baseline data collection was made and thereafter the brachial nerves stimulated at the next higher frequency, 1.6 Hz. This protocol was repeated a third time with the brachial nerves stimulated at 3.2 Hz. The stimulator was triggered by a sine wave signal from an oscillator.

Animal groups

Two groups of Wistar rats were used: the first group received no drug and the brachial nerve stimulation responses acted as controls; the second group received rilmenidine, 100 $\mu g\ kg^{-1}$ i.v. in 0.3 ml saline immediately after the first collection of baseline data, and 15 min later, the second collection was carried out which represented the basal measurement for the brachial nerve stimulation protocol. At the end of the first set of brachial nerve stimulations, a second dose of rilmenidine was given, 200 $\mu g\ kg^{-1}$ i.v. in 0.3 ml saline, and 15 min later after the baseline collection was made another stimulation protocol was repeated.

Two complementary groups of SHRSP were used. The first acted as controls and the second received the two cumulative doses of rilmenidine as described above for the Wistar rats.

Data analysis

The renal nerve activity was amplified by means of an optically isolated amplifier (Grayden, Electronics, Birmingham) with a gain of 100,000 and high- and low-pass filters set at 0.1 and 1.0 kHz, respectively. Both blood pressure and renal nerve activity were displayed on a dual channel oscilloscope and stored on video tape after digitisation with a pulse code modulator. At the same time, signals from the blood pressure, renal nerve and oscillator channels were relayed to an Apple Macintosh (Centris) computer and digitised by means of an A/D converter (National Instruments NB-M10-16H). A data acquisition program written in LabVIEW software was used for on-line analysis to display mean blood pressure and heart rate. In addition, the renal sympathetic nerve activity was rectified, integrated every 1 s and a mean value calculated over the 3.5 min collection period for each variable. Thirty minutes after the animal had been killed, background noise activity was recorded in the renal nerve channel at rest and during the highest rate of brachial nerve stimulation and the values subtracted from the basal and stimulation values, respectively.

Power-spectrum generation

During the final minute of the collection periods a 1 min high frequency sampling of blood pressure, renal nerve activity and the triggering sine wave was taken and stored onto the hard disc for off-line processing. The 1 min recording was dividied into two halves and 1024 points from each segment were passed through a Hanning smoothing window to minimize 'end-leakage' which may result from a finite length of data collection and possible lack of symmetry. The power spectrum for each variable was generated and the relative amount of energy in the renal nerve and blood pressure signal at each frequency, from 0 to 10 Hz in 0.1 Hz increments, was calcu-

lated. Several variables were derived from the power spectral analysis. The total power in the spectrum was derived from the area under the curve, from 0 to 10 Hz and is a measure of the total energy in the signal. The power at heart rate frequency was derived by determining the frequency of the maximum peak in the blood pressure recording, which was taken at heart rate frequency. The power in the area of the renal nerve spectra which coincided with the heart rate frequency, ±0.1 Hz, was taken as the absolute power at heart rate frequency and the percentage power at heart rate was calculated as a proportion of the total power. This reflects the degree of activity generated by the baroreceptor reflex arc. Cross-correlation analysis between the blood pressure and renal nerve spectra was performed in order to generate coherence and phase relationships (Davis & Johns, 1995). Coherence assesses how the two signals correlate with each other with a value of 1 indicating a direct relationship, whereas a value of 0 indicates no relationship. The phase difference indicates how the two signals relate to each other in the frequency domain, with a difference of 0 radians demonstrating synchrony of the two signals, and a value of 3.142 radians (II) corresponding to a reciprocal relationship between the two signals. It is possible that the phase difference may allow interpretation of how the central nervous system organises sympathetic outflow in relation to both the basal level of blood pressure and the pressure pulse.

Statistics

The data quoted are mean±s.e.mean values. The integrated renal sympathetic nerve activity presented in the tables was normalised by expressing the renal nerve activity during the stimulation period as a percentage of the control period immediately before stimulation in the absence as well as in the presence of each dose of drug. The data were analysed by two way ANOVA and a Bonferroni/Dunn post hoc test (Super-ANOVA, Abacus, Berkely, California, U.S.A.) between the vehicle- and drug-related groups. The responses for all variables to a given stimulus were calculated as the difference between the control period immediately before stimulation and that obtained during brachial nerve stimulation. Statistical differences were taken when $P<0.05$.

Results

The influence of rilmenidine on blood pressure, heart rate normalised and integrated renal nerve activity in the Wistar rats is shown in Table 1. It is clear that rilmenidine caused a dose-related depression in both blood pressure and heart rate ($P<0.05$ for both variables at each dose) while integrated nerve activity was 4.86±0.8 mV s^{-1} in the vehicle group and was decreased by the low (4.86±0.80 to 4.53±0.71 mV s^{-1}) and high (4.56±0.77 to 3.78±0.70 mV s^{-1}) doses of rilmenidine, respectively ($P<0.05$). Stimulation of the brachial nerves (Table 1) led to significant ($P<0.05$) increases in blood pressure and heart rate at each frequency level, although there was only a very shallow relationship between stimulation frequency and response while integrated renal sympathetic nerve activity was increased only at the highest stimulation frequency ($P<0.05$). In spite of the lower blood pressure and heart rate following rilmenidine 100 μg kg^{-1} (Table 1), brachial nerve stimulation again caused significant ($P<0.05$) rises in blood pressure and heart rate which were similar in both pattern and magnitude to those obtained in the absence of the drug. Moreover, in the presence of this low dose of rilmenidine, it was only the highest frequency of brachial nerve stimulation which raised integrated renal sympathetic nerve activity ($P<0.01$). The second dose of rilmenidine resulted in even lower basal levels of blood pressure and heart rate, but during brachial nerve stimulation there were increases in blood pressure and heart rate ($P<0.05$) while integrated renal sympathetic nerve activity actually fell at 0.8 Hz but was raised at the two higher stimulation frequencies ($P<0.05$). These responses matched very closely those obtained in the absence of the drug.

The cardiovascular responses to brachial nerve stimulation in the SHRSP in the absence and presence of rilmenidine are presented in Table 2. In the SHRSP, blood pressure and integrated renal nerve activity were higher and heart rate lower than that of the Wistar rats (Table 1). Stimulation of the brachial nerves in the SHRSP (Table 2) caused significant (all $P<0.05$) increases in blood pressure at the two higher stimulation frequencies while heart rate was unchanged. Under these conditions, integrated nerve activity was elevated at each stimulation frequency ($P<0.01$). Rilmenidine 100 μg kg^{-1} (Table 2) resulted in lower levels of blood pressure, heart rate and integrated renal sympathetic nerve activity (10.96±1.70 versus 10.28±1.60 mV s^{-1}) (all $P<0.05$), but when the brachial nerves were stimulated there were increases in blood pressure and heart rate at each frequency level ($P<0.05$) while integrated renal sympathetic nerve activity was raised only by the two higher stimulation frequencies. These responses were similar in pattern and size to those obtained in the absence of the drug. The second dose of rilmenidine, 200 μg kg^{-1} (Table 2) resulted in further decreases in blood pressure and heart rate ($P<0.05$ for both) as well integrated renal sympathetic nerve activity, which fell from 10.22±1.44 immediately before drug administration to 8.27±1.74 mV s^{-1} ($P<0.001$) 15 min later. However, when the brachial nerves were stimulated, there were increases in blood pressure at all stimulation frequencies ($P<0.05$) while heart rate was raised significantly only at 0.8 and 1.6 Hz, the variability at 3.2 Hz being such that significance was not achieved; integrated renal sympathetic nerve activity only increased at 1.6 and 3.2 Hz, comparable in all respects to the responses obtained in the absence and presence of the low dose of rilmenidine.

Figure 1 shows a recording of a renal nerve signal obtained from a Wistar rat before and 15 min after 200 μg kg^{-1} rilmenidine. Figure 1a and b is a raw nerve signal which shows a high signal to noise ratio and good pulsatility, but in the presence of rilmenidine the pulsatility seems smaller. Figure 1c

Table 1 Effect of brachial nerve stimulation on blood pressure, heart rate and integrated renal nerve activity in Wistar rats

			Stimulation frequencies		
		Control	*0.8 Hz*	*1.6 Hz*	*3.2 Hz*
Vehicle	BP (mmHg)	101±4	115±3**	124±7**	120±5**
	HR (beats min^{-1})	435±14	446±14***	459±15**	458±12***
	Integrated RSNA (%)	100±0	95±4	97±5	106±6*
Rilmenidine 100 μg kg^{-1}	BP (mmHg)	89±5	108±5***	111±6***	110±6**
	HR (beats min^{-1})	409±17	436±13**	438±21**	441±22*
	Integrated RSNA (%)	100±0	102±4	103±4	117±4**
Rilmenidine 200 μg kg^{-1}	BP (mmHg)	75±7	91±7***	99±7***	104±6**
	HR (beats min^{-1})	369±20	390±24*	406±24***	425±22***
	Integrated RSNA (%)	100±0	85±6	111±4*	124±8*

Data shown are means±s.e.mean. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 2 Effect of brachial nerve stimulation on blood pressure, heart rate and integrated renal nerve activity in hypertensive rats (SHRSP)

			Stimultaion frequencies		
		Control	*0.8 Hz*	*1.6 Hz*	*3.2 Hz*
Vehicle	BP (mmHg)	158±6	117±8	186±10*	195±9*
	HR (beats min^{-1})	370±11	378±11	387±12	395±13
	Integrated RSNA (%)	100±0	104±2**	106±1***	110±2***
Rilmenidine 100 $\mu g\ kg^{-1}$	BP (mmHg)	137±9	156±10**	170±7***	170±8***
	HR (beats min^{-1})	318±16	333±14*	350±8*	354±10*
	Integrated RSNA (%)	100±0	99±2	105±1**	105±1**
Rilmenidine 200 $\mu g\ kg^{-1}$	BP (mmHg)	114±13	128±13***	149±13**	154±10*
	HR (beats min^{-1})	298±23	303±23*	316±24*	331±19
	Integrated RSNA (%)	100±0	100±2	114±9*	120±13**

Data shown are means±s.e.mean. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

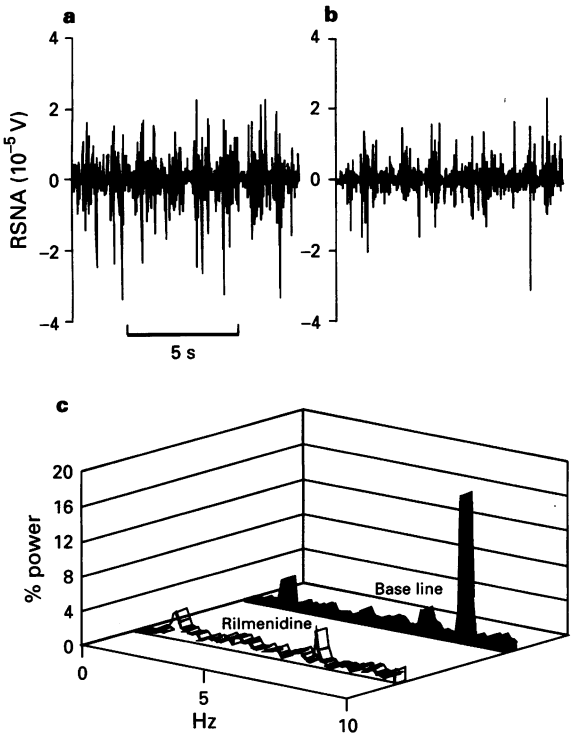

Figure 1 A renal nerve recording from a Wistar rat given as a raw signal (RSNA-renal sympathetic nerve activity) (a) in the absence (basal) and (b) in the presence of rilmenidine (200 $\mu g\ kg^{-1}$). (c) The power spectra generated from the signal, % power being the percentage of power occurring at each frequency (Hz) with total power being taken as the area under the curve from 0–10 Hz.

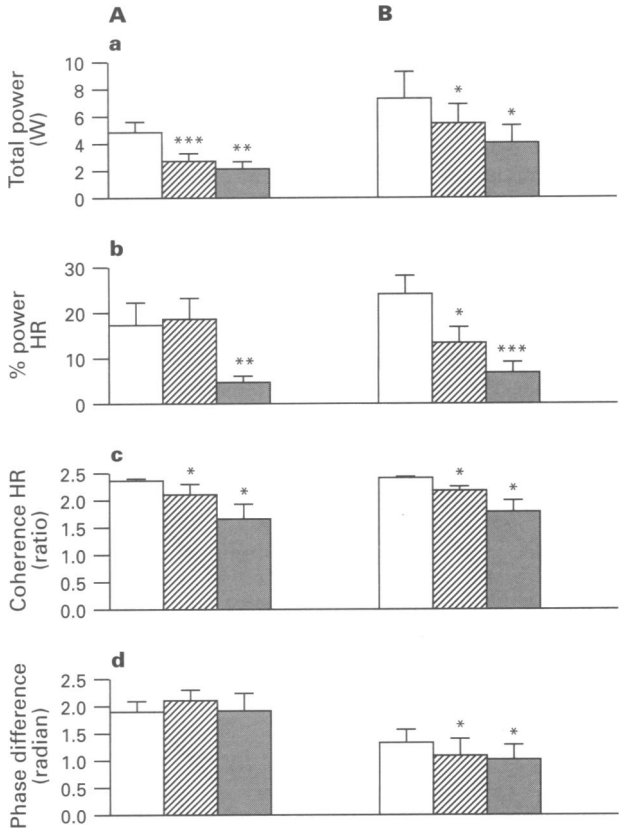

Figure 2 The basal renal nerve power spectral parameters given as (a) total power (in watts), (b) percentage power occurring at heart rate (% power HR), (c) coherence within the signal at heart rate (coherence HR) and (d) the phase difference between the renal nerve and blood pressure signals in (A) Wistar rats and (B) SPSHR. Open columns represent vehicle group; hatched columns, 100 $\mu g\ kg^{-1}$ rilmenidine; solid columns, 200 $\mu g\ kg^{-1}$ rilmenidine. *$P<0.05$; **$P<0.01$ and ***$P<0.001$: comparison between the value obtained before and 15 min after rilmenidine or vehicle administration.

shows the power spectra generated from these two signals and it can be seen that in the basal state there is a marked peak at approximately 8 Hz which corresponds to heart rate frequency with other small peaks at lower frequencies one of which, between 1–2 Hz, corresponds to respiration frequency. In the presence of rilmenidine there was an inhibition in the power occurring at heart rate frequency but relatively little change at any other frequency.

The basal levels of the power spectral characteristics of renal nerve activity in the groups of Wistar and SHRSP and the influence of rilmenidine are shown in Figure 2. The total power and the percentage power at heart rate in the renal nerve signal were significantly higher (both $P<0.05$) in the group of SHRSP compared with the Wistar group by approximately 10–20% and, although the coherence in the signal was similar, the phase difference in the signal was significantly ($P<0.05$) lower in the SHRSP. In the Wistar rats, 100 $\mu g\ kg^{-1}$ rilmenidine decreased total power of the renal nerve signal by approximately 30% ($P<0.001$), while the percentage power occurring at the heart rate frequency did not change. There was a 15% decrease ($P<0.05$) in coherence in the signal but the phase relationships were unaltered. Following the second dose of rilmenidine (Figure 2), there were further reductions in total power ($P<0.01$), % power at heart rate frequency (of approximately 60%; $P<0.01$) and coherence (about 15%; $P<0.05$) while the phase difference was not altered. It was evident in the SHRSP (Figure 2) that 100 $\mu g\ kg^{-1}$ rilmenidine decreased total power and % power at heart rate by approximately 25–45% ($P<0.05$ for both), while there were reductions in both the coherence and phase differences (both

$P<0.05$) for about 10%. The second dose of rilmenidine given to the SHRSP (Figure 2) caused further reductions in all variables which were more marked in total power and percentage power at heart rate, of 54% and 70% ($P<0.05$) compared to the untreated levels, but less in regards to coherence and phase differences, of 25% and 30% ($P<0.05$), respectively.

Figure 3 shows the responses of the renal sympathetic nerve activity parameters to brachial nerve stimulation in Wistar rats alone and in the presence of rilmenidine. Brachial nerve stimulation in the animals given vehicle induced marked increases ($P<0.001$) in total power to the same extent (two to three fold) at all levels of stimulation. Although the first dose of rilmenidine had decreased the total power in the signal (Figure 3), there were again large increases during brachial nerve stimulation ($P<0.01$) which were smaller in absolute, but not percentage terms compared with the responses obtained in the absence of the drug. Following the second dose of rilmenidine, brachial nerve stimulation again caused large increases in total power ($P<0.001$) which were very comparable to those obtained when the drug was either not present or given at 100 $\mu g\ kg^{-1}$. The pattern of response in the percentage power at heart rate was somewhat different in that brachial nerve stimulation induced significant reductions (all $P<0.01$) of between 50–60% at all frequency levels. Furthermore, these decreases in % power at heart rate still occurred, and to the same degree compared to the altered baseline, at the low dose of rilmenidine. These responses were abolished at the high dose of rilmenidine which appeared to be due to the low baseline values of this parameter induced by the drug being unable to be reduced further.

The effect of brachial nerve stimulation on the renal sympathetic nerve activity parameters in SHRSP are shown in Figure 4. It can be seen that stimulation of the brachial nerves increased total power in the signal at each frequency level by approximately 50% ($P<0.05$) which was similar to the response observed in the Wistar rats. Administration of the 100 $\mu g\ kg^{-1}$ dose of rilmenidine into the SHRSP had little effect on the basal level of total power, and hardly influenced the magnitude of the response obtained to each frequency of brachial nerve stimulation. Although the 200 $\mu g\ kg^{-1}$ dose of rilmenidine decreased the basal level of total power in the signal, brachial nerve stimulation still caused an increase ($P<0.05$) in total power at each stimulation frequency. These responses obtained in the SHRSP in the presence of rilmenidine were very comparable to those observed in the Wistar given the drug. Brachial nerve stimulation at all frequencies in the absence of the drug caused small, approximately 15–30%, increases ($P<0.05$) in the percentage power at heart rate which was in marked contrast to the reductions obtained in the Wistar rats. Rilmenidine 100 $\mu g\ kg^{-1}$ decreased the basal levels of percentage power at the heart rate, but all frequencies of brachial nerve stimulation given to the SHRSP still caused marked rises in this variable of between 100–200% ($P<0.01$) to levels comparable to those produced during stimulation in the absence of the drug. Following the second dose of rilmenidine into the SHRSP, brachial nerve stimulation at all frequencies was still able to cause large increases in the percentage power at heart rate ($P<0.001$), reaching levels similar to those achieved when the drug was not present, but again this response was markedly different from that obtained in Wistar rats.

Discussion

The intention of this study was twofold. Firstly, to examine in detail whether rilmenidine affected the pattern of activity within the renal nerve signal, which is generated centrally and might be susceptible to modulation by the drug. That is, could the drug cause a change in the proportion of the energy within the renal nerve signal at the heart rate frequency, which measures the linkage between the arterial baroreceptors and sympathetic outflow; or alter the phase differences, which estimates the differential between the peaks in the pressure wave and sympathetic activity, or the coherence of the signals which are generated, all of which are dependent upon a complex integration within the central nervous system. Secondly, the issue was addressed as to whether reflex activation of the renal nerves and the power spectral composition following stimulation of somatosensory receptors, might be modified by a central action of the rilmenidine.

Renal nerve activity recorded in these preparations showed good pulsatility that corresponded with the blood pressure wave, and a slower wave corresponding to respiration frequency (1 Hz) with a large signal to noise ratio that made it suitable for fast Fourier transformation to generate a power spectrum. The power spectra generated had marked peaks

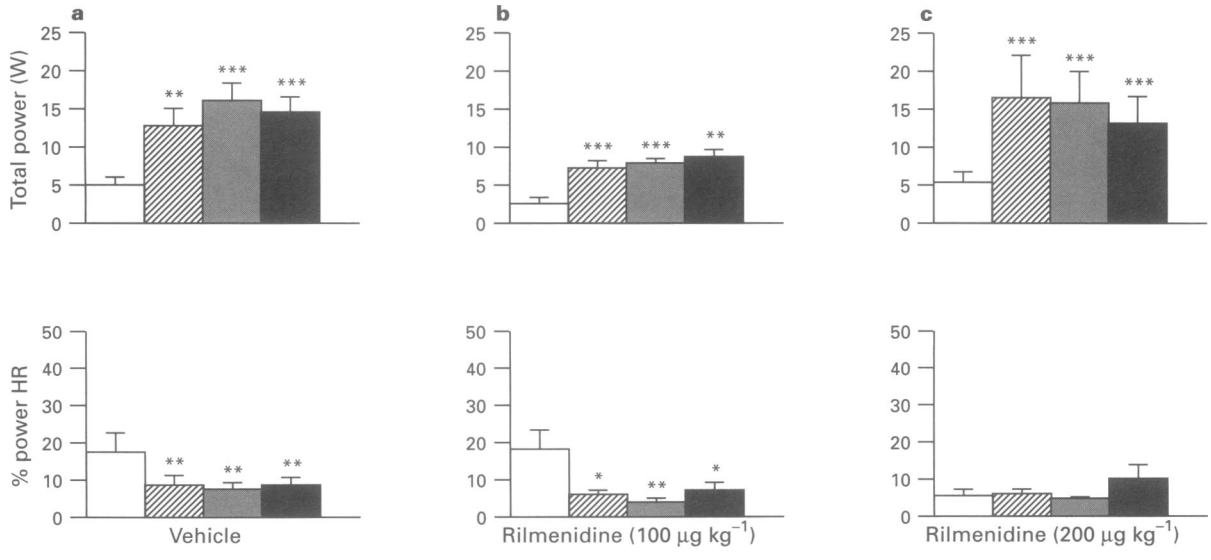

Figure 3 The response of total power and percentage power occurring at heart rate (% power HR) before (open columns) and during brachial nerve stimulation, 0.8 (hatched columns), 1.6 (checked columns) and 3.2 Hz (solid columns), in groups of Wistar rats receiving (a) saline (vehicle) or rilmenidine (b) 100 or (c) 200 $\mu g\ kg^{-1}$. The control value shown is the average value of all control values immediately before each stimulation. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$: comparisons are between the control value immediately before and the value obtained during each stimulation frequency.

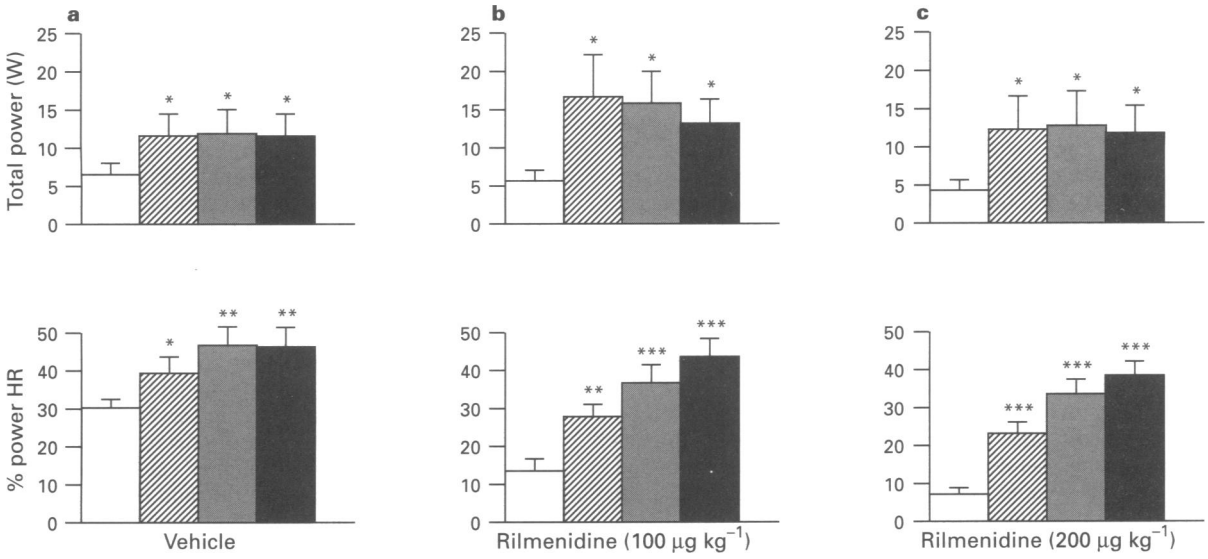

Figure 4 The response of total power and percentage power occurring at heart rate (% power HR) before (open columns) and during brachial nerve stimulation at 0.8 (hatched columns), 1.6 (checked columns) and 3.2 Hz (solid columns), in groups of SHRSP rats receiving (a) saline (vehicle) or rilmenidine (b) 100 or (c) 200 $\mu g\,kg^{-1}$. The control value shown is the average value of all control values immediately before each stimulation. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$: comparisons are between the control value immediately before and the value obtained during each stimulation frequency.

corresponding to heart rate frequency with smaller peaks at lower frequencies, at least one of which was related to respiration frequency. From these spectra it was possible to determine the coherence in the signal and the phase difference between the renal sympathetic nerve and the blood pressure signals and the latter parameter may indirectly indicate the level of baroreceptor control of the nerve activity. There were differences in the spectral pattern of the renal nerve activity in the SHRSP in that not only was total power higher compared with the Wistar rats, but the percentage of that power occurring at heart rate was elevated, 25–30% in the SHRSP against 15–25% in the Wistar. Perhaps more importantly the phase differences were smaller, ie 1.5 radians in the SHRSP and 2.0 radians in the Wistar. What this means is that in the hypertensive rats the increase in renal nerve activity took place at a higher point in the blood pressure wave compared to the Wistar. Whether this change might reflect the baroreflex being reset to a higher pressure in these hypertensive rats remains to be determined, although it is known that the baroreceptor reflex is reset in hypertension.

Stimulation of the somatic afferent nerves of the brachial nerve plexus in this way leads to a generalised increase in sympathetic outflow, as reflected by the rise in both blood pressure and heart rate, and the sympathetic outflow to the kidney at the higher rates of stimulation. This is most likely caused by depolarization of high threshold afferent fibres carried by the brachial nerves which originate from the skin and muscle of the forelimbs (Davis & Johns, 1994b) and is a situation in which the baroreflex is likely to be inhibited (Coote, 1978). Under these conditions in the normotensive rats there were marked changes in the pattern of renal nerve activity; firstly, the total power within the signal rose, due to an increase in nerve activity occurring at the stimulus frequency (Davis & Johns, 1995), but importantly, the power at heart rate frequency fell markedly. This effect of brachial nerve stimulation on the power spectral pattern has been obtained previously (Davis & Johns, 1995). There are major differences in the SHRSP in the way in which renal nerve activity is generated and modulated by somatosensory stimulation. Thus, brachial nerve stimulation led to an increase in blood pressure and total power in the nerve signal, as seen in the normotensive rats, but, more importantly, in the SHRSP the percentage power at heart rate increased markedly, in stark contrast to the large reduction observed in the Wistar rat. This has been described in our previous studies (Davis & Johns, 1992), although the underlying reasons are unclear. However, one possibility is that in the Wistar, the baroreflex modulation of renal sympathetic nerve activity is sensitive to the increased somatosensory input whereby the pattern of sympathetic outflow becomes modulated to the frequency at which the brachial nerves are stimulated and less affected by the heart rate frequency. In the SHRSP it would seem that the generation of sympathetic outflow in relation to the heart rate frequency was much more rigidly fixed and insensitive to modulation by somatosensory stimulation (Zhang & Johns, 1995a, b). Indeed, as indicated earlier, there were similar increases in the power occurring with increased frequency of stimulus in both Wistar and SHRSP, as previously reported (Davis & Johns, 1992; 1995).

Administration of rilmenidine systemically led to dose-dependent reductions in blood pressure and heart rate in both Wistar and SHRSP that were comparable to those obtained previously in these rat strains, albeit at slightly different doses and modes of administration, eg, continuous infusion versus bolus doses (Sannajust *et al.*, 1992; Kline & Cechetto, 1993). Interestingly, integrated nerve activity appeared less sensitive to the compound in that the proportional decreases in nerve activity were only about half those in blood pressure; this is a general indicator of sympathetic outflow, in both normotensive and hypertensive animals. Nevertheless, the renal sympatho-inhibition observed at this dose was comparable to that obtained by others in anaesthetized and conscious normotensive and hypertensive rats (Sannajust *et al.*, 1992; Kline & Cechetto, 1993) and rabbits (Szabo *et al.*, 1993) and has been considered by some to be due to activation of imidazoline receptors by rilmenidine in the rostral venterolateral medulla of the brain. However, this remains a contentious area as two recent studies by Uban *et al.* (1995a, b) in the rabbit, have been unable convincingly to distinguish pharmacologically between the cardiovascular effects of a highly selective α_2-adrenoceptor agonist (ie UK14304) and monoxidine and rilmenidine. In terms of the power spectral patterns, there were dose-related effects which were more evident at the higher dose of rilmenidine. Thus, in both normotensive and hypertensive rats there was a decrease in total power in the signal together with a small loss of coherence which was probably related to the decrease in integrated nerve activity. A feature of note was that, in the Wistar the phase difference either rose slightly at

the lower dose, or was unaffected by the second dose of the compound. Thus, in spite of the fall in blood pressure, the relationship between the pressure at which the renal nerve activity began to increase was the same, ie there was an acute resetting of the baroreceptors to operate at the lower pressure. By contrast, in the SHRSP, the phase difference actually decreased and became shorter at a time when blood pressure fell, that is, the point along the blood pressure wave at which nerve activity began to increase was probably close to that occurring before the drug was given. This suggests that in these animals there was less ability to reset the baroreceptor reflex. Thus, although rilmenidine caused an overall decrease in sympathetic outflow, there was no evidence that it changed or disturbed the basic way in which the renal nerve activity was generated in either the normotensive or hypertensive animals.

It was clear that, in spite of the depressed basal blood pressure and heart rates induced by the rilmenidine, stimulation of the brachial nerves still caused reflex increases in blood pressure and heart rates the magnitudes of which, although slightly less in absolute terms, were similar in proportionate terms to those observed in the absence of the drug. Thus, no persuasive evidence was obtained that rilmenidine influenced in any way the ability of the somatosensory reflexes to cause the vasopressor or tachycardia responses in either the normotensive or hypertensive animals.

In terms of the responses of the power spectral characteristics, it was apparent that rilmenidine had little influence on the somatosensory-induced responses, particularly at the lower dose. Thus, in the Wistar rats although basal levels of both total power and % power at heart rate were lower, the increases in total power and reciprocal decreases in % power at heart rate were still very evident when the brachial nerves were activated. However, at the high dose of the compound, the percentage power at heart rate frequency was so low initially that it was likely that it would not be possible to measure a further decrease, therefore this variable remained unchanged during the brachial nerve stimulation. Moreover, in the SHRSP, in the presence of both low and high doses of rilmenidine, there were increases in the total power and % power at heart rate during the somatosensory challenge and from both the magnitudes and patterns of the response there was no evidence that they were in any way altered by the presence of the drug. However, whether a suppression of the responses could have been obtained if higher doses of rilmenidine had been used was not explored.

In summary, this study set out to examine how the putative imidazoline I_1-receptor agonist, rilmenidine might affect both the basal level of renal sympathetic nerve activity and its reflex activation following somatosensory nerve activation in normal and hypertensive rats. Rilmenidine caused dose-related decreases in blood pressure, heart rate and integrated renal nerve activity, total power and % power at heart rate. The modulation of the renal nerve spectral pattern by the somatosensory challenge was depressed at the higher dose in the Wistar rats but in the hypertensive rats there was no indication that rilmenidine interfered in any way with the reflex control of renal nerve activity.

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