Original Article

Actions of rilmenidine on neurogenic hypertension in BPH/2J genetically hypertensive mice

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Objective: BPH/2J hypertensive mice have an exaggerated sympathetic contribution to blood pressure (BP). Premotor sympathetic neurons within the rostroventrolateral medulla (RVLM) are a major source of sympathetic vasomotor tone and major site of action of the centrally-acting sympatholytic agent, rilmenidine. The relative cardiovascular effect of rilmenidine in BPH/2J versus normotensive BPN/3J mice was used as an indicator of the involvement of the RVLM in the sympathetic contribution to hypertension in BPH/2J mice.

Methods: BPH/2J and BPN/3J mice were pre-implanted with telemetry devices to measure BP in conscious unrestrained mice. Rilmenidine was administered acutely (n=7-9/group), orally for 14 days at a wide range of doses (n=5/group) and also infused intracerebroventricularly for 7 days (n=6/group).

Results: Acute intraperitoneal rilmenidine induced greater depressor and bradycardic responses in BPH/2J than BPN/3J mice (P_{strain}<0.01). Both responses were reduced by atropine pre-treatment, with the remaining hypotensive effect being small and comparable between strains (P_{strain}=1.0). This suggests that vagally induced reductions in cardiac output were responsible for the hypotension. Chronic intracerebroventricularly infused rilmenidine reduced BP from baseline marginally in BPH/2J mice during the dark (active) period (-6.5±2mmHg, P=0.006). Chronic orally administered rilmenidine (1-12mg/kg/day) also had minimal effect on 24-hour BP in both strains (P>0.16).

Conclusion: The sympathetic vasomotor inhibitory effect of rilmenidine is minimal in both strains and similar in hypertensive BPH/2J and BPN/3J mice. Thus hypertension in BPH/2J mice is not likely mediated by greater neuronal activity in the RVLM and agents such as rilmenidine would be an ineffective treatment for this form of neurogenic hypertension.

Key words: Hypertension; rilmenidine; BPH/2J mice; sympathetic nervous system; rostroventrolateral medulla.

INTRODUCTION

Genetically hypertensive BPH/2J mice are thought to have a neurogenic form of hypertension primarily based on ganglion blockade being capable of abolishing the hypertension and greater mid frequency mean arterial pressure (MF MAP) power in BPH/2J mice, which is indicative of greater sympathetic nervous system (SNS) activity [1]. The hypertension in BPH/2J mice has been suggested to be similar to that of 'white-coat' hypertensive patients based on the SNS hyper-responsivity, exaggerated circadian related BP surges and cardiovascular hyper-reactivity to stressful situations that is apparent in both BPH/2J mice and white coat hypertensive patients [1-6]. Early studies reported abnormal catecholamine levels in the brain of BPH/2J mice compared with control mice indicating that the central nervous system (CNS) may play a role in the hypertension [7, 8]. More recently brain imaging studies have identified differences in neuronal activity in autonomic cardiovascular regulatory brain regions using both cytochrome oxidase and Fos as markers of neuronal activity [1, 9]. Taken together these studies suggest that hypertension in BPH/2J mice may be of central origin and principally involving greater sympathetic vasomotor tone.

Premotor sympathetic neurons located within the rostroventrolateral medulla (RVLM) are recognized as a major region regulating the SNS contribution to the cardiovascular regulation [10-12]. Hypertension in spontaneously hypertensive rats (SHR) is reported to be neurogenic particularly early in the development of the hypertension prior to structural changes [13] due to higher sympathetic activity involving the RVLM [14, 15]. SHR are also very responsive to centrally acting antihypertensive drugs such as clonidine and rilmenidine [16]. Whilst there are peripherally

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mediated BP effects of these drugs [17, 18], the main antihypertensive effect is reportedly due to central sympatholytic actions [19].

Rilmenidine in particular is an agonist at imidazoline and α-adrenergic receptors and its central hypotensive effects has been shown to be mainly, if not entirely through actions at the RVLM [20]. The main evidence for this comes from studies in conscious rabbits indicating that small doses of antagonists into the RVLM block the hypotensive actions of systemic rilmenidine [19]. Additionally there is also evidence from the marked hypotensive effect of intra-RVLM injections of low dose rilmenidine in rats. [21, 22]. To our knowledge there has been limited assessment of the specific actions of rilmenidine in the RVLM of mice. However intraRVLM injection of moxonidine, a similar imidazoline/ alpha2 agonist, produce marked depressor responses in anaesthetised WT mice which were of comparable magnitude as that induced by 4th ventricle injections of rilmenidine in WT mice [23, 24].

Whether the RVLM contributes to the hypertension in BPH/2J mice is unresolved. Studies by Davern and colleagues showed that activation of the RVLM in BPH/2J mice, as assessed by Fos immunohistochemistry, occurs during the dark (active) period of the mouse nocturnal cycle but the level of activation is similar to BPN/3J mice [1]. By contrast in BPH/2J mice the very much greater level of activation in the forebrain regions such as the hypothalamus and also the amygdala [1] suggest that the hypertension in BPH/2J is more related to areas involved in the cardiovascular response to aversive stress [25] and may therefore involve activation of the SNS without involving the bulbospinal presympathetic pathways via the RVLM [26]. If this is correct, treatment with rilmenidine would be relatively ineffective as its major site of action is the RVLM. Thus the aim of the present study is to determine whether the neurogenic hypertension in BPH/2J mice is responsive to treatment with rilmenidine which would likely indicate a major role of the pre-sympathetic neurons in the RVLM.

The acute cardiovascular response to rilmenidine was determined both with and without vagal inhibition, to delineate the sympatholytic from vagal excitatory effects of rilmenidine [27]. Rilmenidine was also chronically infused directly into the lateral ventricle of the brain to determine the effect on BP and stress reactivity whilst aiming to minimise the peripheral effects of rilmenidine on BP [28]. The sympatholytic effect of rilmenidine treatment was assessed using spectral analysis to assess BP variability in the mid frequency (MF) range as an indicator of SNS activity. We also determined the chronic hypotensive effect of orally administered rilmenidine at a wide range of dose. Together these studies provide a comprehensive evaluation of the effect of rilmenidine in hypertensive BPH/2J and normotensive BPN/3J mice.

Methods

Experiments were performed on male normotensive (BPN/3J, n=38) and hypertensive mice (BPH/2J, n=41). The experiments were approved by the Alfred Medical Research Education Precinct Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for Scientific Use of Animals.

Preliminary surgeries

ICV cannula implantation

BPH/2J (n=15) and BPN/3J (n=12) mice undergoing chronic central infusion (Study 2) first underwent surgery to implant a 30G intracerebroventricular (ICV) guide cannula, implanted under Ketamine (Ketalar, Pfizer)/Xylazine (Ilium Xylazil-20, Smithfield, Australia)/Atropine (Sigma, StLouis, USA) mixture, 100, 10, and 1.2mg/kg respectively. A computer aided Leica Angle Two Mouse stereotaxic apparatus directed placement of the cannula tip at 0.5mm posterior from bregma, 1.2mm lateral from the midline and 2.2mm ventral to the skull surface. The cannula was secured in place with M1.0 cheese-head stainless steel screws (Mirofasteners, Melbourne, Victoria, Australia) and dental cement (Vertex, Zeist, Netherlands). SP45 tubing attached to the cannula by SP10 tubing was tunneled along the back of the neck to allow connection of a minipump (Alzet model 1002, DURECT Corporation, Cupertino, CA, US) at a later stage. Anaesthesia was reversed with 0.2mg/kg Atipamezole HCl (Antisedan, Pfizer). Mice were allowed 14 days to recover prior to subsequent telemetry surgery.

Telemetry probe implantation

All mice (Study 1, 2 and 3) underwent surgery to implant BP telemetry transmitters (model TA11PA-C10; Data Sciences International, St Paul, Minnesota, USA) under isoflurane open circuit anesthesia (5% induction and 1.5-2% maintenance). The catheter of the telemetry device was inserted into the carotid artery and the transmitter probe was positioned subcutaneously along the right flank [29]. The mice were housed individually in a room with 12:12 hour light-dark cycle (1am-1pm light) with access *ad libitum* to water and mouse chow (Specialty Feeds, Glen Forrest, Western Australia, 19% protein, 5% fat, 5% fiber, 0.2% sodium).

Preliminary recordings

After 10 days recovery from radiotelemetry surgery, all mice underwent an initial continuous 48-hour recording of systolic (SAP), diastolic (DAP) and calculated mean arterial pressure (MAP), heart rate (HR) and locomotor activity measured in freely moving mice in their home cage. The recordings were sampled at 1000 Hz using an analog-to-digital data acquisition card (National Instruments 6024E) as described previously [30]. The beat-to-beat arterial pressure and HR were detected on line and analyzed later using a program written in Labview [31]. All subsequent cardiovascular and locomotor recordings for study 1, 2 and 3 were conducted in the same manner.

Study 1: Acute peripheral rilmenidine treatment

During the dark (active) period (>2 hours after dark onset) cardiovascular parameters of BPN/3J (n=7-9) and BPH/2J mice (n=7-9) were measured 30 minutes before and after intraperitoneal (i.p.) injections of either 1.5mg/kg rilmenidine, 2mg/kg atropine (Sigma,St Lois, MO, USA) and saline. Additionally rilmenidine was also administered 30 minutes following pre-treatment with atropine. Acute drug doses used in the present study were based on those reported to have hypotensive effectiveness [32, 33]. All drugs were freshly prepared in 0.9% saline each day and the effect of each drug or drug combination were assessed on separate days.

Study 2: Chronic ICV and subcutaneous and administration

Preliminary dose finding study

To identify an effective central dose with minimal peripheral effects, a preliminary cumulative ICV or subcutaneous (s.c.) dose response curve to rilmenidine dihydrogen phosphate (Servier, Neuilly, France; 6, 10 and 20μg) was performed on separate days with BPH/2J mice (n=3). The dose of rilmenidine selected for chronic infusion (15μg/hr) was chosen to mimic a similar steady state response as the 20μg ICV bolus (as determined by the preliminary dose response study).

Main study

Baseline measurements of cardiovascular parameters and locomotor activity were recorded for 48 hours followed by behavioral tests (detailed below) performed in the two subsequent days. Hypertensive BPH/2J and normotensive BPN/3J mice were administered either Ringer's solution (vehicle, n=6/group) or 15μg/hour rilmenidine (n=6/group) infused via an osmotic minipump (0.22ul/hr). Osmotic pumps were implanted subcutaneously under isoflurane open circuit anesthesia (5% induction and 1.5-2% maintenance) through a small incision between the scapula. Following 7 days of s.c. infusion, cardiovascular parameters and locomotor activity were recorded for 48 hours and the behavioral tests repeated. To infuse rilmenidine or Ringer's solution into the lateral ventricle mice underwent a final minor surgery to connect a subcutaneously implanted minipump via SP45 tubing to the pre-implanted ICV guide cannula. Following 7 days of ICV infusion, cardiovascular parameters and locomotor activity were recorded for another 48 hours and behavioral tests were repeated.

Behavioral tests

After 48-hour cardiovascular recordings at baseline and during *s.c.* and ICV infusion, mice were exposed to aversive behavioral stimuli performed on two separate days during the light period when the animals were inactive as described previously [3, 34]. Restraint was conducted on the first day and dirty cage-switch stress was performed on the second day. Restraint stress was performed by guiding the mouse into a cylindrical plexiglass restrainer with a sliding back plate to confine the animal for 60 minutes. Dirty cage-switch stress involved removing the mouse from its home cage and placing it for 60 minutes in a cage previously occupied by another male mouse.

Cardiovascular variability and the cardiac baroreceptor sensitivity

During chronic minipump administration of rilmenidine the beat-to-beat data from 48-hour recordings were analyzed separately to calculate power spectra using a program written in Labview [31]. The auto- and cross-power spectra were calculated for multiple overlapping (by 50%) segments of MAP and HR using a Fast Fourier transform as adapted for conscious mice [35]. The cardiac baroreflex sensitivity was estimated as the average value of the transfer gain in the frequency band between 0.3 and 0.5 Hz [35]. Baroreflex slope was considered significant if the coherence between MAP and HR across several overlapping segments in the analyzed frequency band was >0.4. Data from the light (inactive) period and dark (active) period with low locomotor activity were chosen (usually 4 from each circadian period) from 48 hour recordings minimizing the influence of physical activity.

Light-Dark BP surge analysis

The continuous 48-hour cardiovascular measurements recorded at baseline and during s.c and ICV rilmenidine, were averaged to hourly values fitted to a double logistic curve using an equation described previously [36]. Using this analysis the average range of the BP surge (mmHg); average rate of rise in BP (mmHg/hour); and the duration of the rise in BP (hours) was determined for the BP surge leading from the light to the dark period in these nocturnal mice, which is equivalent to the morning surge in BP displayed in humans.

Study 3: Oral rilmenidine treatment (14 days)

BPH/2J and BPN/3J mice (n=5/group) were treated with rilmenidine at 1 and 2 or 6 and 12mg/kg/day with the dose range based upon previous studies in rats [16, 37, 38]. Each mouse received two rilmenidine doses added to the drinking water and water alone in a random order cross over design. Rilmenidine doses were adjusted according to the volume ingested during the previous period every 5 days during treatment. After 14 days of treatment, cardiovascular parameters and locomotor activity were recorded for another 48 hours. No washout period was allowed between the three treatments received by each mouse because pharmacokinetic data suggests rilmenidine is rapidly removed from the plasma [38] and as such no carry over effect was expected on the cardiovascular measurements carried out more than 14 days after changing the treatment .

Statistical Analysis

Cardiovascular data were expressed as mean ± standard error of the mean (SEM). The data were

analyzed by multi-factor, nested split-plot analysis of variance (ANOVA), which allowed for within animal and between animal contrasts.[39] A combined residual was used that pooled the between and within animal variance as described previously [40]. A probability of $P<0.05$ was considered significant. Effect of treatment "treat" represents the effect relative to baseline. Effect of strain represents the difference between BPN/3J and BPH/2J mice.

Results

Study 1: Acute effect of i.p. rilmenidine treatment

Rilmenidine alone

MAP, HR and locomotor activity during the 30 minute control period prior to treatment were greater in BPH/2J mice than BPN/3J mice ($P_{strain}<0.01$). Rilmenidine induced marked depressor responses from baseline in both strains ($P_{treat}<0.001$) which were 87% greater in BPH/2J mice (n=8) compared with BPN/3J mice (n=9, $P_{strain}=0.004$, Figure 1A). There were also large bradycardic responses in both strains ($P<0.001$ for both), which were 42% greater in the BPH/2J compared with BPN/3J mice ($P_{strain}=0.008$). Additionally, rilmenidine induced reductions in locomotor activity in both strains but more so in BPH/2J mice ($P_{treat}<0.01$, $P_{strain}<0.001$).

Atropine pre-treatment prior to rilmenidine

Rilmenidine administration following atropine pre-treatment induced mild hypotensive responses in both strains ($P_{treat}<0.01$, Figure 1A) which were comparable between strains ($P_{strain}=1.0$). However depressor responses induced by rilmenidine following atropine treatment were markedly lower compared with rilmenidine treatment alone in BPH/2J mice (n=8, $P_{treat}<0.001$) but not BPN/3J mice (n=7, $P_{treat}=0.08$). Atropine pre-treatment attenuated the bradycardic response to rilmenidine alone in BPH/2J ($P_{treat}=0.006$) but not BPN/3J mice ($P_{treat}=0.3$). Atropine pre-treatment attenuated the reductions in locomotor activity caused by rilmenidine treatment in BPH/2J mice ($P_{treat}=0.001$) but not BPN/3J mice ($P_{treat}=0.6$).

Atropine alone

Atropine treatment induced a small rise in BP in BPH/2J mice (n=7, $P_{treat}<0.001$) but not BPN/3J mice (n=8, $P_{treat}=0.2$, $P_{strain}=0.09$, Figure 1B). Atropine induced similar tachycardic responses in both strains ($P_{treat}<0.001$, $P_{strain}=0.9$). Locomotor activity was reduced by atropine treatment in BPN/3J mice ($P=0.008$) but not BPH/2J.

Control (vehicle)

Vehicle administration induced a slight increase in MAP in BPH/2J mice (4.9±1.5mmHg, $P_{treat}=0.02$) but not BPN/3J mice ($P_{treat}=0.9$) although responses were similar between strains ($P_{strain}=0.22$, Figure 1B). Following vehicle administration HR returned to baseline in BPN/3J ($P_{treat}=0.9$) but remained slightly elevated in BPH/2J mice ($P_{treat}=0.003$, $P_{strain}=0.02$). Locomotor activity returned to baseline following vehicle treatment in BPH/2J mice ($P_{treat}=0.6$) but tended to remain elevated in BPN/3J mice ($P_{treat}=0.05$).

Study 2: Chronic ICV and subcutaneous and administration

Preliminary dose finding study

Acute ICV injection of 6-10μg of rilmenidine reduced MAP by 3-5 % from baseline in BPH/2J mice, but i.p. injections of the same doses induced comparable responses ($P_{route}=0.07$, Figure 2A). Treatment with 20μg rilmenidine ICV, induced a 13% reduction in MAP which was over 3 times greater than the response induced by 20μg of rilmenidine injected i.p. ($P_{route}<0.001$, Figure 2A). The hypotensive effect of treatment with 20μg rilmenidine ICV was observed to last between 60-90 minutes (Figure 2B).

Effect of chronic minipump infused rilmenidine on cardiovascular and locomotor measurements

24-hour average MAP was 24% greater in hypertensive BPH/2J than normotensive BPN/3J mice ($P_{strain}<0.001$), whilst HR was 29% greater ($P_{strain}<0.001$) and locomotor activity was 1.8 fold greater (n=6/group, $P_{strain}<0.001$). Following 7 days of ICV or s.c. infusion of rilmenidine (15μg/hr) there was no change to MAP or HR during the light (inactive) period in BPN/3J or BPH/2J mice (n=6/group, Figure 3). However, ICV administration of rilmenidine reduced MAP by 5% in BPH/2J ($P_{treat}=0.006$) during the dark (active) period but not in BPN/3J mice ($P_{treat}=0.5$). Subcutaneously administered rilmenidine reduced MAP by 5% in both BPN/3J and BPH/2J mice ($P_{treat}<0.05$). Importantly there was no strain by treatment interaction for either ICV ($P_{Interaction}=0.9$) or s.c. infused rilmenidine ($P_{Interaction}=1.0$). During the dark (active) period ICV administration of rilmenidine reduced HR in BPN/3J and BPH/2J mice ($P<0.01$), whilst s.c. treatment only reduced HR in BPN/3J mice ($P=0.01$). There was no strain by treatment interaction for either ICV ($P_{Interaction}=0.8$) or s.c. administered rilmenidine ($P_{Interaction}=0.9$). Neither ICV nor s.c. administration of rilmenidine affected day-time or night-time activity levels in either strain ($P_{treat}>0.1$, Figure 3).

Following 7 days of ICV or s.c. infusion of Ringer's solution there was no change to MAP ($P_{treat}>0.07$), HR ($P_{treat}>0.09$) or locomotor activity ($P_{treat}>0.6$) during the light (inactive) period or dark (active) period in BPN/3J or BPH/2J mice (n=6/group, Figure 4).

Cardiovascular variability and cardiac baroreflex sensitivity

In the dark (active) period, MF MAP power was 2.4 fold greater in BPH/2J compared with BPN/3J mice ($P_{strain}<0.001$, n=6/group, Table 1 & Table S1) whilst baroreflex gain tended to be greater in BPN/3J than BPH/2J mice ($P_{strain}=0.06$). Chronic ICV and s.c. infusion of rilmenidine both decreased MF MAP power in BPH/2J mice ($P_{treat}<0.002$). By contrast neither s.c. nor ICV administration of rilmenidine effected MF MAP power in BPN/3J mice ($P_{treat}=1.0$). ICV administration of

rilmenidine increased gain in BPH/2J mice only (P_{treat}<0.001), whilst peripheral rilmenidine treatment only tended to increase gain in BPH/2J mice (P_{treat}=0.06).

During the light (inactive) period MF MAP power (P_{strain}=1.0), baroreflex gain (P_{strain}=1.0) and MF HR power (P_{strain}=0.2) were similar in BPN/3J and BPH/2J mice (n=6/group, Table 1 & Table S2). MF MAP power was not affected by chronic s.c. (P_{treat}>0.2) or ICV infusion of rilmenidine (P_{treat}>0.6) in either strain. Both s.c. and ICV administration of rilmenidine increased baroreflex gain in BPH/2J mice (P_{treat}<0.03) but not BPN/3J mice (P_{treat}=1.0).

Light-dark BP surge analysis

At baseline, the difference in MAP from the light to dark plateau (MAP range) was 2.4 fold greater in BPH/2J than BPN/3J mice (P_{strain}<0.001, Table 2). The rate at which this surge in BP took place was comparable between strains (MAP rate of rise (ROR); P_{strain}=1.0) as was the time taken for the surge to take place (Duration; P_{strain}=0.7). Vehicle treatment, whether infused s.c. or ICV did not influence MAP range, ROR or duration in either strain (P_{treat}>0.07, Table 2). Likewise, subcutaneous rilmenidine infusion also did not change MAP range, ROR or duration in either strain (P_{treat}>0.1). ICV administration of rilmenidine did not affect the BP range in either strain (P_{treat}>0.1). However, it did cause a marked reduction in the MAP ROR in both strains (P_{treat}<0.05). Additionally ICV rilmenidine treatment caused a marked increase in duration of the BP surge in BPH/2J mice only (P_{treat}=0.02).

Cardiovascular response to behavioral tests

Dirty cage-switch

Dirty cage-switch stress elicited a rise in BP and locomotor activity in both strains (Figure S1, n=6/group) which were greater in BPH/2J than BPN/3J mice (P_{strain}<0.001), whereas the rise in HR was 23% greater in BPN/3J compared with BPH/2J mice (P_{strain}=0.009). Subcutaneous infusion of rilmenidine had minimal effect on the pressor, tachycardic and locomotor activity response to dirty cage switch stress in both strains, except for an attenuation of the tachycardic response in BPH/2J mice (P_{treat}=0.04). By contrast ICV infusion of rilmenidine attenuated the pressor and tachycardic responses in both strains (P_{treat}<0.05) and also attenuated the locomotor activity response in BPH/2J mice (P_{treat}=0.03).

Restraint

One hour of restraint stress elicited sustained pressor responses in both strains (n=6/group, Figure S2) which were 41% greater in BPH/2J compared with BPN/3J mice (P_{strain}<0.001). By contrast the tachycardic response was less in BPH/2J mice (P_{strain}<0.001). ICV and *s.c.* rilmenidine had minimal effect on the pressor, tachycardic and locomotor activity responses to restraint in both strains except ICV rilmenidine which attenuated the tachycardic response in BPN/3J mice (P_{treat}=0.002).

Study 3: Effect of chronic oral rilmenidine

Baseline MAP was 19% greater in BPH/2J compared with BPN/3J mice (P_{strain}<0.001), whilst HR was 26% greater (P_{strain}<0.001) and locomotor activity was over 2 fold greater (P_{strain}=0.01). Chronic orally administered rilmenidine at doses 1, 2, 6 and 12mg/kg/day, had no effect in either strain on MAP (P_{treat}>0.2), HR (P_{treat}>0.2) or locomotor activity (P_{treat}>0.7, Figure 5).

Discussion

The aim of the present study was to establish whether the neurogenic hypertension in BPH/2J mice is responsive to rilmenidine treatment as this would be indicative of a contribution from the pre-sympathetic neurons in the RVLM. The initial findings showed acute i.p. rilmenidine produced a marked ~35mmHg hypotension in BPH/2J compared with ~20 mmHg in BPN/3J mice. This dose also produced profound bradycardia which was markedly greater in BPH/2J (-320 b/min) compared with BPN/3J (-220 b/min). If these finding were considered alone, this may be interpreted as indicating BPH/2J mice have higher sympathetic vasomotor tone. However the greater depressor and bradycardic response to rilmenidine in BPH/2J compared with BPN/3J mice was abolished by atropine, likely by preventing a reduction in cardiac output. Without these vagal excitatory effects of rilmenidine, the acute hypotensive response to rilmenidine was comparable between strains. The remaining hypotension after atropine, was minimal and similar in both strains, most likely reflecting the sympatho-inhibitory actions of rilmenidine. Indeed chronic central infusion of rilmenidine reduced BP only marginally during the dark (active) period in BPH/2J mice and also had little effect in BPN/3J mice. The limited hypotensive effect is not likely due to an insufficient dose of rilmenidine because two week oral administration at a wide range of doses (1-12 mg/kg/day) also had similarly little effect on BP in both strains. Thus the present study provides evidence from peripheral, central, acute and chronic treatments in a wide range of doses that the sympathetic vasomotor inhibitory actions of rilmenidine treatment is modest at best and is similar in both strains of mice. This contrasts the large hypotensive response we have previously reported using ganglion blockade, which was greater than 50 mmHg in BPH/2J and 30mmHg in BPN/3J mice [1]. This treatment abolished the difference between strains and was the primary evidence for suggesting that the hypertension in BPH mice was of neurogenic origin. Since the primary site of action of rilmenidine is at imidazoline and α2 adrenoceptors within the RVLM [19], these findings together suggest that sympathetic outflow influenced by the RVLM is not contributing to BPH/2J hypertension. We conclude that hypertension in BPH/2J is likely to arise from sympathetic activity driven from regions other than the RVLM.

Effect of chronic central rilmenidine infusion

Previous studies have shown that vagal excitatory effects become less prominent with chronic treatment [27, 41] likely due to central α_2-adrenoceptors desensitization [38]. Thus to avoid these and other acute effects on the heart [18] as well as other peripheral actions of rilmenidine[42], the main focus of the present study involved determining the central sympatholytic hypotensive effects by central infusion of rilmenidine. The initial acute dose finding study in BPH/2J mice determined an ICV dose of rilmenidine that produced a greater hypotensive effect than when given peripherally. Central infusion of the chosen dose of rilmenidine for 7 days only produced mild hypotensive responses during the dark (active) period in BPH/2J mice. Furthermore this was not greater than the response in BPN/3J mice and did not reduce daytime BP in either strain. This contrasts with findings showing comparatively larger hypotensive effects in SHR treated chronically with rilmenidine [16, 38]. The pressor response to restraint stress was not influenced by rilmenidine treatment in either strain. This is consistent with the ability of rilmenidine to preserve phasic BP responses including those associated with stress [43] and also suggests that vascular reactivity was unaltered by rilmenidine treatment. ICV infusion of rilmenidine slightly attenuated the pressor response to dirty cage-switch stress but this was likely related to the mild reduction of the extreme locomotor activity surge associated with this particular stressor. Rilmenidine infusion markedly reduced the rate of the rise (ROR) in BP from light (inactive) to dark (active) period in both strains, a parameter which is positively associated with SNA [44], indicating there may be some influence of rilmenidine on SNA in these mice. Moreover rilmenidine infusion also resulted in a reduction in night-time MAP power in the MF range only in BPH/2J mice, which indicates a reduction in SNS activity. Rilmenidine treatment also increased baroreflex gain exclusively in BPH/2J mice, which is consistent with the effect of rilmenidine in mild essential hypertensive patients [45]. The reduction in MF MAP power to similar levels observed in normotensive BPN/3J mice indicates that chronic rilmenidine treatment does appear to effectively reduce vasomotor SNS activity. However despite the apparent sympatholytic effect, rilmenidine treatment does not result in an overtly greater hypotensive effect in BPH/2J mice.

In the final study we used an alternate route of administration and a wide range of doses, performed to ensure that any central sympathoinhibitory actions of rilmenidine would be revealed. Doses between 1 and 12 mg/kg/day of rilmenidine were administered via drinking water for two weeks. This route of administration is known to have hypotensive effects in SHR [16]. However chronic oral treatment for two weeks did not influence 24-hour BP in either strain, despite being administered at doses ranging from below to above dosages shown to effectively lower BP in SHR [16, 37, 38]. As such it appears that chronic administration of rilmenidine, whether administered centrally or peripherally (orally or by s.c. minipump) does not induce marked hypotensive responses in either strain. Chronic delivery of rilmenidine is not widely reported in normotensive rats, but SHR are reported to respond with 8-15% decrease in BP depending on dose and route of administration [16, 38], therefore the minimal hypotensive effect of rilmenidine in hypertensive BPH/2J mice is unexpected.

The expectation that rilmenidine should induce hypotensive responses of approximately similar magnitude as the hypotension caused by ganglion blockade is reasonable given our previous findings in other normotensive species such as the rabbit. In these previous studies, pentolinium reduces BP by 20 mmHg in conscious normotensive rabbits [46] while rilmenidine has a maximal effect of approximately 17mmHg [47]. However centrally acting antihypertensive drugs such as rilmenidine are usually slightly less effective than ganglionic blockade as they have specific regional hemodynamic effects [48, 49] partially due to incomplete vasomotor sympatho-inhibition and different regional sensitivity [50]. Furthermore centrally acting antihypertensive agents, can induce vasoconstriction peripherally by post synaptic vascular α-adrenoceptor activation [42] and centrally they are capable of inducing pressor effects at the level of the hypothalamus [51-53], such that in some circumstances the hypertensive effects may counteract the hypotensive effects [54, 55].

Importantly the sympatholytic effect of rilmenidine is shown to be mediated by premotor neurons located within the RVLM [56-58], and as such rilmenidine treatment does not inhibit sympathetic outflow from other sympathetic premotor regions projecting directly to the spinal cord. The very modest hypotensive effect of rilmenidine we observed were similar in both strains of mice suggesting that outflow from this region is not inherently greater in BPH/2J mice. Therefore SNS over-activity in BPH/2J mice may be mediated through one of the other sympathetic premotor regions, such as the PVN, A5 noradrenergic cell group, caudal raphe or ventromedial medulla [59]. Interestingly BPH/2J mice are not the only model of sympathetically mediated hypertension where the RVLM does not appear to be involved. Sympathetic premotor neurons within the PVN but not the RVLM were shown to be more important to the hypertensive response in a conditioned fear model [60]. Additionally low dose AngII induced hypertension in rabbits, which is accompanied by elevated RSNA, is shown to be associated with greater long term neuronal activation of the PVN but not the RVLM [61]. This AngII induced hypertensive model may be particularly similar to the BPH/2J mice since our recent findings also suggests that the hypertension in BPH/2J mice is caused by a greater contribution of the systemic renin angiotensin

system (RAS) caused by renal hyper-innervation and greater sympathetically induced renin synthesis [62]. Indeed our own previous findings indicate greater neuronal activity in the PVN but not the RVLM of BPH/2J compared with BPN/3J mice during periods of arousal [1].

Conclusion and perspectives

The present study provides a comprehensive characterization of the cardiovascular effect of rilmenidine in mice, which to our knowledge is limited to a small number of studies which have only assessed the acute cardiovascular response to rilmenidine administration [24, 63]. Importantly the findings of present study indicate that, marked vagal enhancement is contributing substantially to the hypotensive effect in mice when administered acutely, a factor which should be considered in future studies in mice. Furthermore this study showed that the sympathetically mediated hypertension in BPH/2J mice is not markedly inhibited by the sympatholytic actions of rilmenidine, whether administered acutely, chronically, centrally or peripherally at a wide range of doses. As such, the greater contribution of the SNS in BPH/2J mice is not likely mediated by over-activity of premotor neurons within the RVLM. Consequently, other sympathetic premotor regions [59] such as the PVN may play a greater role in mediating sympathetic over-activity likely driven from forebrain regions in BPH/2J mice. Since rilmenidine did not abolish the hypertension in BPH/2J mice, this suggests it may be a less effective treatment option in certain neurogenic forms of hypertension.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Davern PJ, Nguyen-Huu T, La Greca L, Head GA. Role of the sympathetic nervous system in Schlager genetically hypertensive mice. Hypertension. 2009; 54 (4):852-9.
2. Davern PJ, Head GA. Role of the medial amygdala in mediating responses to aversive stimuli leading to hypertension. Clin Exp Pharmacol Physiol. 2011; 38:136-43.
3. Davern PJ, Jackson KL, Nguyen-Huu T, La Greca L, Head GA. Cardiovascular responses to aversive and non-aversive stressors in Schlager genetically hypertensive mice. Am J Hypertens. 2010; 23 (8):838-44.
4. Smith PA, Graham LN, Mackintosh AF, Stoker JB, Mary DA. Sympathetic neural mechanisms in white-coat hypertension. J Am Coll Cardiol. 2002; 40 (1):126-32.
5. Head GA, Chatzivlastou K, Lukoshkova EV, Jennings GL, Reid CM. A novel measure of the power of the morning blood pressure surge from ambulatory blood pressure recordings. Am J Hypertens. 2010; 23 (10):1074-81.
6. Lantelme P, Milon H, Gharib C, Gayet C, Fortrat JO. White coat effect and reactivity to stress: cardiovascular and autonomic nervous system responses. Hypertension. 1998; 31 (4):1021-9.
7. Schlager G, Freeman R, El Seoudy AA. Genetic study of norepinephrine in brains of mice selected for differences in blood pressure. J Hered. 1983; 74 (2):97-100.
8. Denoroy L, Sautel M, Schlager G, Sacquet J, Sassard J. Catecholamine concentrations in discrete brain nuclei and sympathetic tissues of genetically hypertensive mice. Brain Res. 1985; 340 (1):148-50.
9. Strazielle C, Lalonde R, Thifault S, Hamet P. Regional brain variations of cytochrome oxidase activity in spontaneously hypertensive mice. Exp Brain Res. 2004; 157 (2):255-64.
10. Bergamaschi CT, Campos RR, Lopes OU. Rostral ventrolateral medulla - A source of sympathetic activation in rats subjected to long-term treatment with L- NAME. Hypertension. 1999; 34 (4):744-7.
11. Colombari E, Sato MA, Cravo SL, Bergamaschi CT, Campos RR, Jr., Lopes OU. Role of the medulla oblongata in hypertension. Hypertension. 2001; 38 (3 Pt 2):549-54.
12. Horiuchi J, Dampney RAL. Dependence of sympathetic vasomotor tone on bilateral inputs from the rostral ventrolateral medulla in the rabbit: role of baroreceptor reflexes. Neurosci Lett. 1998; 248 (2):113-6.
13. Adams MA, Bobik A, Korner PI. Differential development of vascular and cardiac hypertrophy in genetic hypertension. Relation to sympathetic function. Hypertension. 1989; 14:191-202.
14. Matsuura T, Kumagai H, Kawai A, Onimaru H, Imai M, Oshima N, et al. Rostral ventrolateral medulla neurons of neonatal Wistar-Kyoto and spontaneously hypertensive rats. Hypertension. 2002; 40 (4):560-5.
15. Muratani H, Ferrario CM, Averill DB. Ventrolateral medulla in spontaneously hypertensive rats - role of angiotensin-II. Am J Physiol Regul Integr Comp Physiol. 1993; 264:R388-R95.
16. Sannajust F, Julien C, Barres C, Cerutti C, Koenig-Berard E, Sassard J. Cardiovascular effects of rilmenidine, a new alpha 2-adrenoceptor agonist, and clonidine in conscious spontaneously hypertensive rats. Clin Exp Pharmacol Physiol. 1989; 16:837-48.
17. Szabo B, Bock C, Nordheim U, Niederhoffer N. Mechanism of the sympathoinhibition produced by the clonidine-like drugs rilmenidine and moxonidine. Ann N Y Acad Sci. 1999; 881:253-64.
18. Urban R, Szabo B, Starke K. Involvement of peripheral presynaptic inhibition in the reduction of sympathetic tone by moxonidine, rilmenidine and UK 14304. Eur J Pharmacol. 1995; 282 (1-3):29-37.
19. Head GA, Burke SL, Chan CK. Site and receptors involved in the sympathoinhibitory actions of rilmenidine. J Hypertens Suppl. 1998; 16:S7-S12.
20. Head GA, Burke SL. I_1 Imidazoline receptors in cardiovascular regulation : the place of rilmenidine. Am J Hypertens. 2000; 13 (6):89S-98S.

21. Zhang J, Abdel-Rahman AA. The hypotensive action of rilmenidine is dependent on functional N-methyl-D-aspartate receptor in the rostral ventrolateral medulla of conscious spontaneously hypertensive rats. Journal of Pharmacology & Experimental Therapeutics. 2002; 303 (1):204-10.
22. Li G, Abdel-Rahman AA. Direct evidence for imidazoline (I1) receptor modulation of ethanol action on norepinephrine-containing neurons in the rostral ventrolateral medulla in conscious spontaneously hypertensive rats. Alcohol Clin Exp Res. 2007; 31 (4):684-93.
23. Tolentino-Silva FP, Haxhiu MA, Waldbaum S, Dreshaj IA, Ernsberger P. alpha(2)-adrenergic receptors are not required for central anti-hypertensive action of moxonidine in mice. Brain Res. 2000; 862 (1-2):26-35.
24. Bruban V, Estato V, Schann S, Ehrhardt JD, Monassier L, Renard P, et al. Evidence for synergy between alpha(2)-adrenergic and nonadrenergic mechanisms in central blood pressure regulation. Circulation. 2002; 105 (9):1116-21.
25. Davern PJ, Jackson KL, Nguyen-Huu T, La Greca L, Head GA. Cardiovascular reactivity and neuronal activation to stress in Schlager genetically hypertensive mice. Neuroscience. 2010; 170 (2):551-8.
26. Vianna DM, Carrive P. Cardiovascular and behavioural responses to conditioned fear and restraint are not affected by retrograde lesions of A5 and C1 bulbospinal neurons. Neuroscience. 2010; 166 (4):1210-8.
27. Godwin SJ, Tortelli CF, Parkin ML, Head GA. Comparison of the baroreceptor-heart rate reflex effects of rilmenidine, moxonidine and clonidine. J Auton Nerv Syst. 1998; 72 (2-3):195-204.
28. Szabo B. Imidazoline antihypertensive drugs: a critical review on their mechanism of action. Pharmacol Ther. 2002; 93 (1):1-35.
29. Butz GM, Davisson RL. Long-term telemetric measurement of cardiovascular parameters in awake mice: a physiological genomics tool. Physiol Genomics. 2001; 5 (2):89-97.
30. Jackson KL, Head GA, Morris BJ, Chin-Dusting J, Jones E, La Greca L, et al. Reduced cardiovascular reactivity to stress but not feeding in renin enhancer knockout mice. Am J Hypertens. 2007; 20:893-9.
31. Head GA, Lukoshkova EV, Burke SL, Malpas SC, Lambert EA, Janssen BJ. Comparing spectral and invasive estimates of baroreflex gain. IEEE Eng Med Biol Mag. 2001; 20 (2):43-52.
32. Tibirica E, Feldman J, Mermet C, Monassier L, Gonon F, Bousquet P. Selectivity of rilmenidine for the nucleus reticularis lateralis, a ventrolateral medullary structure containing imidazoline-preferring receptors. Eur J Pharmacol. 1991; 209:213-21.
33. Gross V, Tank J, Obst M, Plehm R, Blumer KJ, Diedrich A, et al. Autonomic nervous system and blood pressure regulation in RGS2-deficient mice. Am J Physiol Regul Integr Comp Physiol. 2005; 288 (5):R1134-42.
34. Davern P, Chen D, Head G, Chavez C, Walther T, Mayorov D. The role of AT1a receptors in cardiovascular reactivity and neuronal activation following aversive stress in mice. Hypertension. 2009; 54 (6):1262-8.
35. Adams DJ, Head (equal first author) GA, Markus MA, Lovicu FJ, van der Weyden L, Köntgen F, et al. Renin enhancer is critical for regulation of renin gene expression and control of cardiovascular function. J Biol Chem. 2006; 281 (42):31753-61.
36. Head GA, Lukoshkova EV, Mayorov DN, Van den Buuse M. Non-symmetrical double logistic analysis of 24 hour blood pressure recordings in normotensive and hypertensive rats. J Hypertens. 2004; 22 (11):2075-85.
37. Cechetto DF, Kline RL. Complementary antihypertensive action of rilmenidine on the pressure-natriuresis relationship and sodium preference in spontaneously hypertensive rats. J Hypertens Suppl. 1998; 16 (3):S13-S7.
38. Monassier L, Greney H, Thomas L, Bousquet P. Chronic treatment with rilmenidine in spontaneously hypertensive rats: Differences between two schedules of administration. Journal of Cardiovascular pharmacology. 2004; 43 (3):394-401.
39. Snedecor GW, Cochran WG. Statistical Methods. 7th ed. Ames, Iowa: Iowa State University Press; 1980.
40. Korner PI, Badoer E, Head GA. Cardiovascular role of the major noradrenergic cell groups in the rabbit: analysis based on 6-hydroxydopamine-induced transmitter release. Brain Res. 1987; 435 (1-2):258-72.
41. Parkin ML, Godwin SJ, Head GA. Importance of imidazoline-preferring receptors in the cardiovascular actions of chronically administered moxonidine, rilmenidine and clonidine in conscious rabbits. J Hypertens. 2003; 21 (1):167-78.
42. Eerdmans PH, Struyker Boudier HA, De Mey JG. Contractile reactivity of isolated resistance arteries after 4 weeks of treatment with rilmenidine, clonidine or hydralazine in spontaneously hypertensive rats. J Hypertens Suppl. 1991; 9 (6):S348-S9.
43. Esler M, Lux A, Jennings G, Hastings J, Socratous F, Lambert G. Rilmenidine sympatholytic activity preserves mental stress, orthostatic sympathetic responses and adrenaline secretion. J Hypertens. 2004; 22 (8):1529-34.
44. Chatzivlastou K. Does the Reactivity of the Sympathetic Nervous System Contribute to the Morning Surge in Blood Pressure? [Honours]. Melbourne: RMIT; 2008.
45. Finta E, Laude D, Alfoldi S, Farsang C, Elghozi JL. Effects of rilmenidine on 24-h rhythmicity of blood pressure and spontaneous baroreflex sensitivity in essential hypertensive subjects. J Hypertens. 2006; 24 (8):1619-25.
46. Burke SL, Evans RG, Moretti J-L, Head GA. Levels of renal and extrarenal sympathetic drive in Ang II-induced hypertension. Hypertension. 2008; 51 (4):878-83.
47. Chan CKS, Head GA. Relative importance of central imidazoline receptors for the antihypertensive effects of moxonidine and rilmenidine. J Hypertens. 1996; 14 (7):855-64.
48. Smits JF, Struyker-Boudier HA. Regional hemodynamic effects of rilmenidine and clonidine in the conscious spontaneously hypertensive rats. Fundam Clin Pharmacol. 1991; 5 (8):651-61.
49. Magorien R, Hermiller J, Unverferth D, Leier C. Regional hemodynamic effects of clonidine in congestive heart failure. J Cardiovasc Pharmacol. 1985; 7 (1):91-6.
50. Ramage AG, Wilkinson SJ. Evidence that different regional sympathetic outflows vary in their sensitivity to the sympathoinhibitory actions of putative 5-HT1A and alpha 2-adrenoceptor agonists in anaesthetized cats. Br J Pharmacol. 1989; 98 (4):1157-64.
51. Imai Y, Nolan PL, Johnston CI. Tachycardic and hypertensive effects of centrally administered clonidine in conscious rats. Clin Exp Hypertens. 1986; 8 (2):239-58.

52. Ebihara H, Kawasaki H, Nakamura S, Takasaki K, Wada A. Pressor response to microinjection of clonidine into the hypothalamic paraventricular nucleus in conscious rats. Brain Res. 1993; 624 (1-2):44-52.
53. Kawasaki H, Nakamura S, Takasaki K. Central a2-adrenoceptor-mediated pressor response to clonidine in conscious, spontaneously hypertensive rats. Jpn J Pharmacol. 1992; 59:321-31.
54. Colombari E, Camargo L, Saad WA, Renzi A, Deluca LA, Menani JV. Cardiovascular effects of central clonidine in conscious rats after hypothalamic lesions. J Auton Nerv Syst. 1992; 40 (1):49-56.
55. Head GA, de Jong W. Differential blood pressure responses to intracisternal clonidine, a-methyldopa, and 6-hydroxydopamine in conscious normotensive and spontaneously hypertensive rats. J Cardiovasc Pharmacol. 1986; 8 (4):735-42.
56. Head GA, Burke SL. Relative importance of medullary brain nuclei for the sympatho-inhibitory actions of rilmenidine in the anaesthetised rabbit. J Hypertens. 1998; 16:503-17.
57. Mayorov DN, Burke SL, Head GA. Relative importance of rostral ventrolateral medulla in sympathoinhibitory action of rilmenidine in conscious and anesthetized rabbits. J Cardiovasc Pharmacol. 2001; 37 (3):252-61.
58. Li G, Wang X, Abdel-Rahman AA. Neuronal norepinephrine responses of the rostral ventrolateral medulla and nucleus tractus solitarius neurons distinguish the I1- from the alpha2-receptor-mediated hypotension in conscious SHRs. J Cardiovasc Pharmacol. 2005; 46 (1):52-62.
59. Strack AM, Sawyer WB, Hughes JH, Platt KB, Loewy AD. A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. Brain Res. 1989; 491 (1):156-62.
60. Carrive P, Gorissen M. Premotor sympathetic neurons of conditioned fear in the rat. Eur J Neurosci. 2008; 28 (3):428-46.
61. Moretti J-L, Burke SL, Davern PJ, Evans RG, Lambert GW, Head GA. Renal sympathetic activation from long-term low-dose angiotensin II infusion in rabbits. J Hypertens. 2012; 30 (3):551-60.
62. Jackson KL, Marques FZ, Watson AMD, Palma Rigo K, Nguyen-Huu T-P, Morris BJ, et al. A novel interaction between sympathetic overactivity and aberrant regulation of renin by mir-181a in BPH/2J genetically hypertensive mice. Hypertension. 2013:in press.
63. Zhu QM, Lesnick JD, Jasper JR, MacLennan SJ, Dillon MP, Eglen RM, et al. Cardiovascular effects of rilmenidine, moxonidine and clonidine in conscious wild-type and D79N alpha(2A)- adrenoceptor transgenic mice. Brit J Pharmacol. 1999; 126 (6):1522-30.

Table and figure legends

Table 1: Average mid frequency mean arterial pressure (MF MAP) power and baroreflex gain during the light and dark periods in BPN/3J and BPH/2J mice at baseline and following peripheral and central infusion of rilmenidine.

	BPN/3J					BPH/2J				
	Baseline	**Peripheral**	***P***	**Central**	***P***	**Baseline**	**Peripheral**	***P***	**Central**	***P***
MF MAP power (0.3 - 0.5 Hz)										
Dark (active) period	0.55 ± 0.07	0.68 ± 0.13	NS	0.68 ± 0.11	NS	1.32 ± 0.26	0.63 ± 0.12	**0.002**	0.60 ± 0.09	**0.002**
Light (inactive) period	0.77 ± 0.09	0.53 ± 0.08	NS	0.86 ± 0.12	NS	0.81 ± 0.09	0.74 ± 0.11	NS	0.98 ± 0.16	NS
Gain (0.5 - 3 Hz)										
Dark (active) period	10.3 ± 1.0	13.5 ± 0.7	NS	11.8 ± 0.9	NS	6.9 ± 1.4	10.4 ± 1.0	NS	14.2 ± 1.4	**<0.001**
Light (inactive) period	11.5 ± 1.0	11.7 ± 0.6	NS	12.5 ± 0.9	NS	10.3 ± 1.3	15.2 ± 1.5	**0.034**	17.1 ± 2.2	**0.002**

P values are probability for comparison between baseline and peripheral or central rilmenidine in each strain. Values are mean ± SEM; $P<0.05$ is considered significant, $P>0.05$ considered non-significance, NS.

Table 2: Average rise (range), rate and duration of rise in mean arterial pressure (MAP) from day to night in BPN/3J and BPH/2J mice at baseline and following peripheral and central infusion of rilmenidine or vehicle.

Vehicle

	BPN/3J Vehicle					BPH/2J Vehicle				
Treatment	Baseline	Peripheral	*P*	Central	*P*	Baseline	Peripheral	*P*	Central	*P*
Range (mmHg)	11 ± 2.4	8.7 ± 2.0	NS	8.5 ± 2.2	NS	30 ± 2.9	28 ± 2.3	NS	31 ± 3.0	NS
Rate (mmHg/hr)	9.1 ± 1.8	8.2 ± 1.9	NS	7.6 ± 2.3	NS	8.4 ± 1.0	6.5 ± 1.3	NS	4.4 ± 1.0	NS
Duration (Hours)	0.9 ± 0.3	0.8 ± 0.2	NS	2.6 ± 1.9	NS	2.4 ± 0.5	3.3 ± 0.7	NS	5.1 ± 0.8	NS

Rilmenidine

	BPN/3J Rilmenidine					BPH/2J Rilmenidine				
Treatment	Baseline	Peripheral	*P*	Central	*P*	Baseline	Peripheral	*P*	Central	*P*
Range (mmHg)	13 ± 1.7	12 ± 1.7	NS	11 ± 0.8	NS	26 ± 3.4	25 ± 2.1	NS	21 ± 2.8	NS
Rate (mmHg/hr)	7.9 ± 2.4	4.3 ± 1.0	NS	3.0 ± 0.5	**0.039**	8.4 ± 1.2	4.7 ± 0.9	NS	2.2 ± 0.2	**0.009**
Duration (Hours)	1.9 ± 0.7	2.8 ± 1.1	NS	2.7 ± 0.6	NS	2.3 ± 0.6	3.7 ± 0.4	NS	5.7 ± 0.5	**0.017**

P values are probability for comparison between baseline and peripheral or central rilmenidine in each strain. Values are mean ± SEM; $P<0.05$ is considered significant, $P>0.05$ considered non-significance, NS.

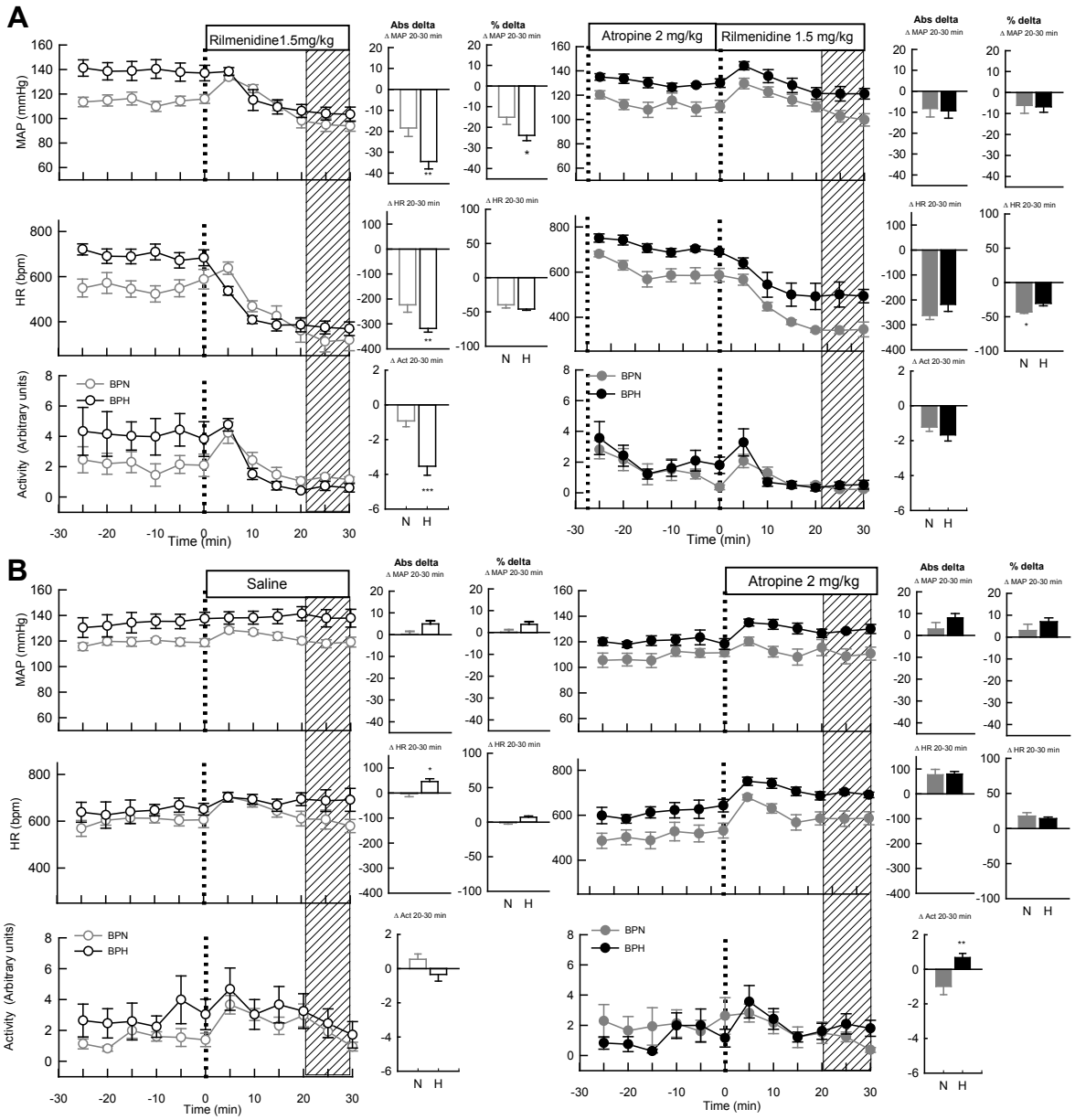

Figure 1: Line graphs represent the MAP, HR, and locomotor activity responses to A; rilmenidine (left) and rilmenidine with atropine pretreatment (right) and B; Saline (left) and atropine alone (right) between BPN/3J (grey; n=7-9) and BPH/2J (black; n=7-8) mice during the dark (active) period. Dashed vertical lines indicate treatment administration. Shaded area represents the period analyzed for comparison of the effect of treatment. Values are mean difference ± SEM. Bar graphs show the absolute (abs) delta and percentage delta from baseline of MAP, HR and locomotor activity from control at 20-30-minute post-injection in BPN/3J (N) and BPH/2J mice (H). Values are mean ± SEM; $^{(*)}$ =0.05, **P*<0.05; ***P*<0.01; ****P*<0.001.

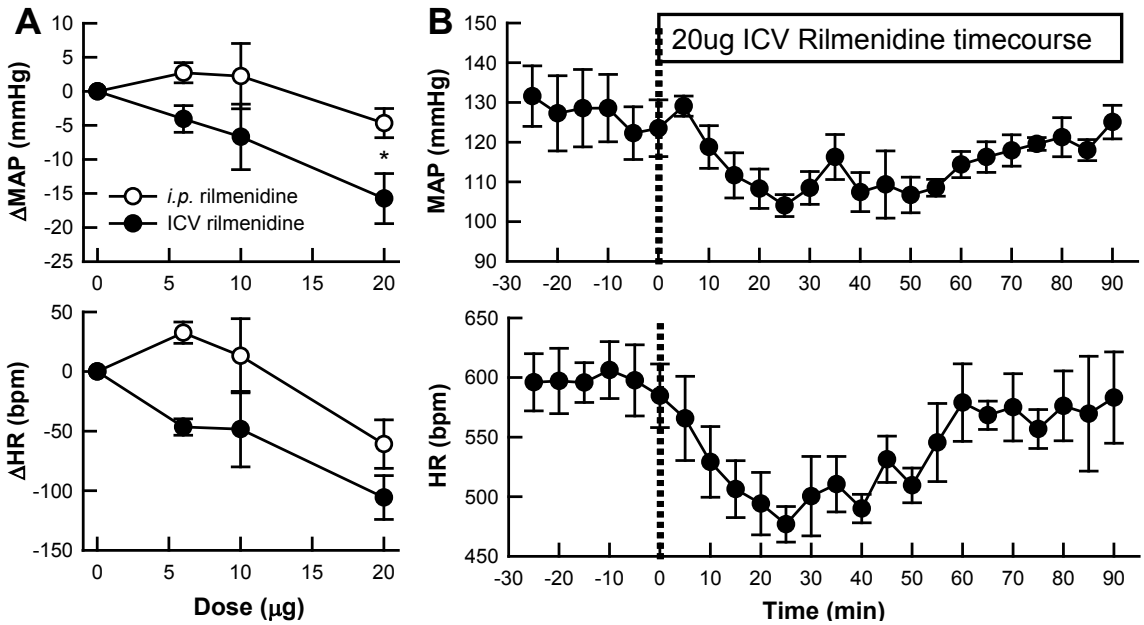

Figure 2:A; Line graphs represents the MAP and HR response to acute ICV (black circles) and i.p. (open circles) injections of rilmenidine at doses of 5,10 and 20ug in BPH/2J mice (n=3). Each point represents the mean change from baseline 20-30 minutes following rilmenidine injection at each dose. Significance refers to between-route difference in response; **P*<0.05. **B;** Line graphs represents the time-course of the MAP and HR response to 20ug ICV dose of rilmenidine. The dotted vertical reference line represents the time-point of ICV rilmenidine injection.

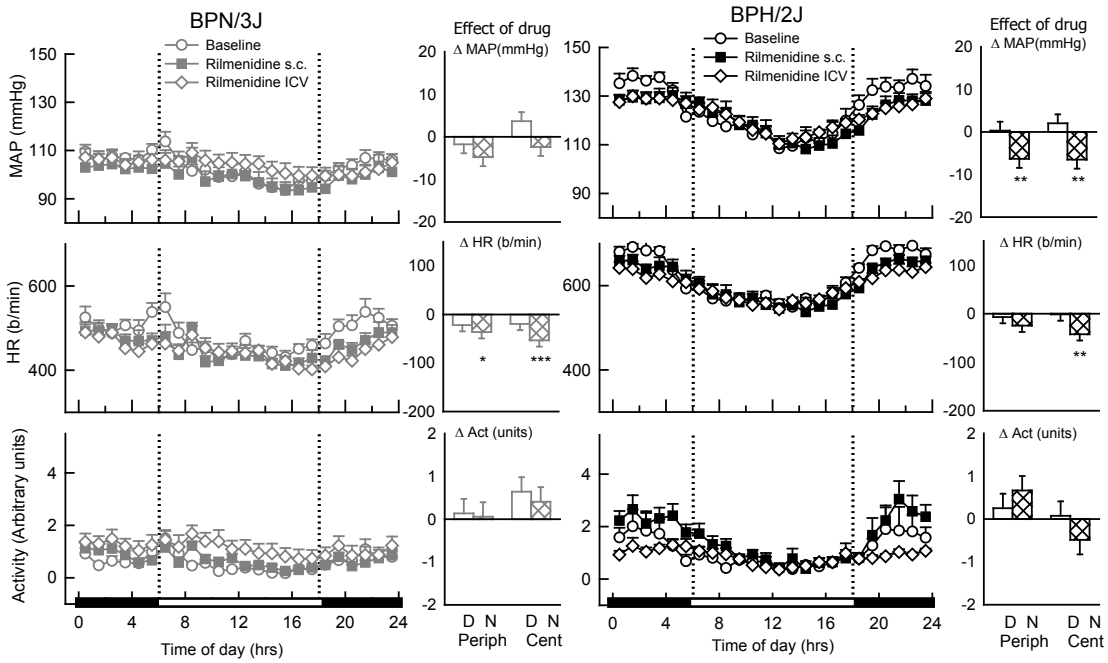

Figure 3: Line graph represents the hourly averaged data showing the circadian variation of MAP (mmHg), HR (beats/min) and locomotor activity (Arbitrary units) during the dark (active, black panels) and light (inactive, white panel) periods in BPN/3J (left, gray) and BPH/2J mice (right, black). Line graphs show pre-treatment baseline (open circles) and average values following peripheral rilmenidine (closed squares) and central rilmenidine (open diamonds) administration. Bar graphs shows mean change ± SEM from baseline induced by peripheral and central rilmenidine treatment during the day (D, unfilled) and night (N, cross hatched) in BPN (middle) and BPH (far right). **P*<0.05; ***P*<0.01; ****P*<0.001 for the probability based on ANOVA

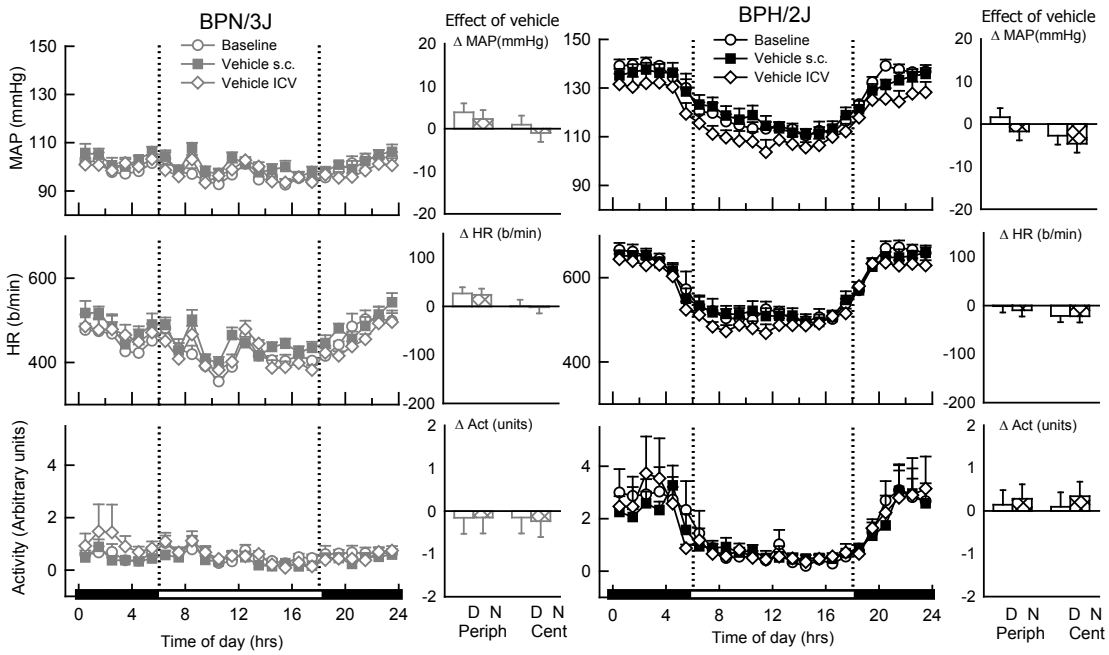

Figure 4: Line graph represents the hourly averaged data showing the circadian variation of MAP (mmHg), HR (beats/min) and locomotor activity (Arbitrary units) during the dark (active, black panels) and light (inactive, white panel) periods in BPN/3J (left, grey) and BPH/2J mice (right, black). Line graphs show pre-treatment baseline (open circles) and average values following peripheral vehicle (closed squares) and central vehicle (open diamonds) administration. Bar graphs shows mean change ± SEM from baseline induced by peripheral and central vehicle treatment during the day (D, unfilled) and night (N, cross hatched) in BPN (middle) and BPH (far right). **P*<0.05; ***P*<0.01; ****P*<0.001 for the probability based on ANOVA

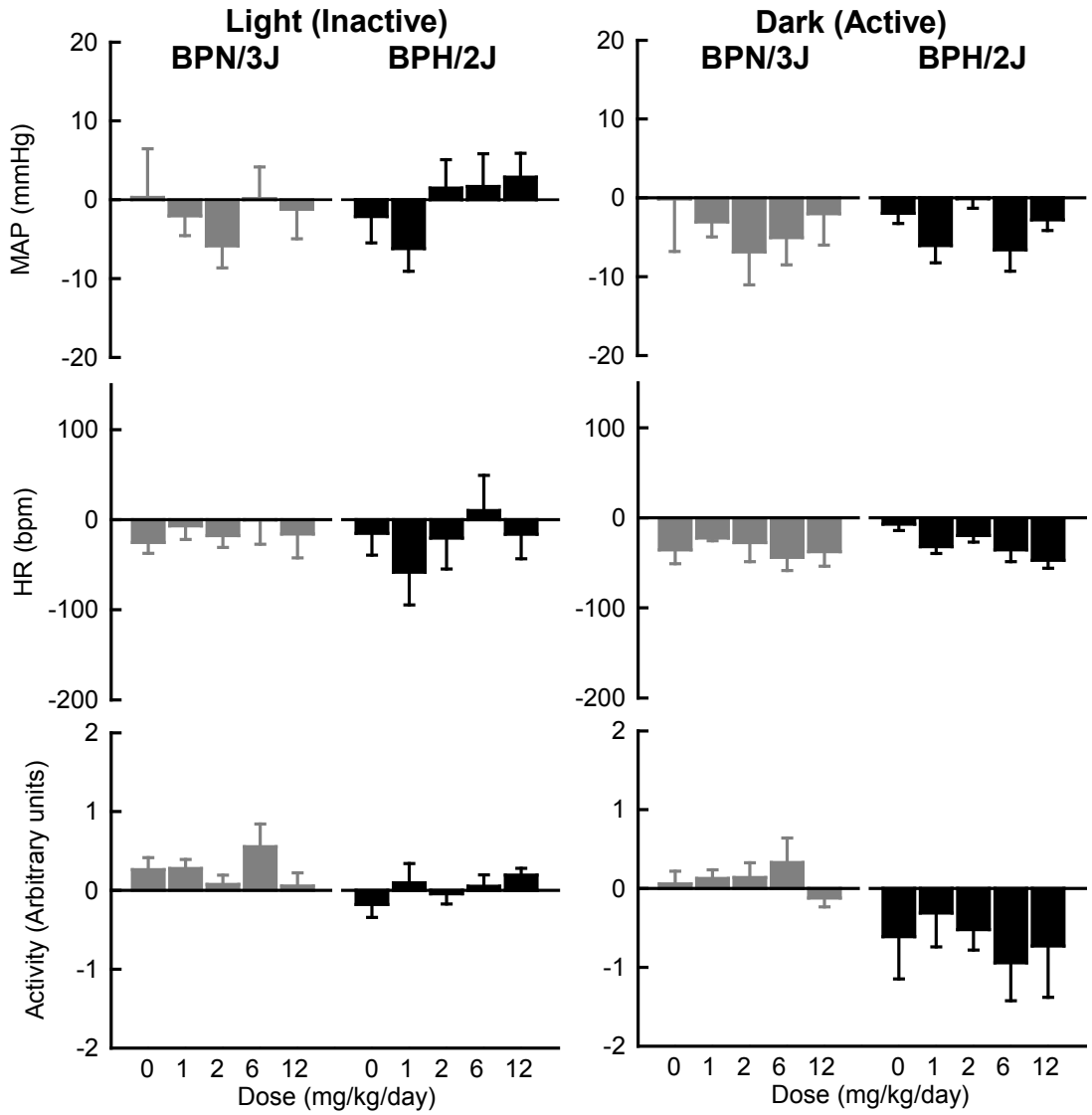

Figure 5: Bar graphs represent change from baseline in MAP (top panel), HR (middle panel) and locomotor activity level (bottom panel) in BPN/3J (grey bars) and BPH/2J mice (black bars) following 14 days of treatment with 0, 1, 2, 6 and 12 mg/kg/day rilmenidine administered orally in the drinking water (Mice are still receiving drug at the time of these measurements). The response is calculated from the two 12 hour light (inactive) periods (left graphs) and two 12 hour dark (active) periods (right) from the 48-hour recordings and are reported as mean difference ± SEM