The present study was designed to investigate the effects of low-dose ketanserin on BPV (blood pressure variability), BRS (baroreflex sensitivity) and organ damage in SHR (spontaneously hypertensive rats). Ketanserin was mixed in rat chow at an estimated dose of 0.1 mg·kg⁻¹·day⁻¹. SHR were treated for 4 months. BP (blood pressure) was then recorded continuously for 24 h in a conscious state. After determination of BRS, rats were killed for organ damage evaluation. It was found that long-term treatment with low-dose ketanserin did not lower BP levels, but significantly decreased BPV, enhanced BRS and reduced organ damage in SHR. Multiple regression analysis showed that the decrease in left ventricular hypertrophy was most closely correlated (or associated) with the increase in BRS, whereas the decrease in aortic hypertrophy was most closely associated with the decrease in diastolic BPV and the amelioration in renal lesion, with the increase in BRS and the decrease in diastolic BPV. In conclusion, low-dose ketanserin produces organ protection independently of its BP-lowering action in SHR, and this organ protection was importantly attributable to the enhancement of BRS and decrease in BPV.

INTRODUCTION

It is well known that BP (blood pressure) level is an important determinant for the end-organ damage in hypertensive patients or hypertensive animals. However, the BP level is certainly not the unique determinant for end-organ damage. Recently, it has been proposed that BPV (BP variability) and BRS (baroreflex sensitivity) may be two important factors determining organ damage in hypertension [1–4]. Ketanserin is an antihypertensive drug with affinity for both 5-HT₂A (where 5-HT is 5-hydroxytryptamine) and α₁ receptors [5,6]. This pharmacological property of ketanserin has aroused the attention of many researchers. Ketanserin not only decreases BP levels, but also reduces BPV and enhances BRS in SHR (spontaneously hypertensive rats) [7]. Interestingly, in an acute study [8], we demonstrated that ketanserin enhanced BRS at a very low dose (0.3 mg/kg of body weight) that had no BP-lowering effect. To distinguish the organ protection produced by BPV reduction and BRS enhancement from that produced by BP reduction, the present study was designed to investigate the effects of long-term treatment with a low dose of ketanserin (0.1 mg·kg⁻¹ of body...
weight·day \(^{-1}\) on BPV, BRS and end-organ damage in SHR.

**METHODS**

**Animals**

Male SHR at 18 weeks of age were provided by the Animal Centre of the Second Military Medical University. The rats were housed with controlled temperature (23–25 °C) and lighting (light from 08:00–20:00 hours; dark from 20:00–08:00 hours), and with free access to food and tap water. All the animals used in this work received humane care in compliance with Institutional Animal Care Guidelines.

**Drug administration**

Ketanserin (Janssen Company) was mixed in the rat chow. The consumption of rat chow containing drugs was as determined previously [7,9]. The content of drugs in the rat chow was calculated according to the chow consumption, and the ingested dose of ketanserin was approx. 0.1 mg·kg\(^{-1}\)·day\(^{-1}\). The control SHR received the same diet without ketanserin. After 4 months of drug administration, BP was recorded over 24 h and BPV was then calculated, and BRS was determined in conscious freely moving rats. Histopathological examinations were performed after BP recording and BRS studies.

**BP measurement**

SBP (systolic BP), DBP (diastolic BP), MAP (mean arterial pressure) and HP (heart period) of SHR were recorded continuously using a technique described previously [9,10]. Briefly, rats were anaesthetized with a combination of ketamine (40 mg/kg of body weight) and diazepam (6 mg/kg of body weight). A floating polyethylene catheter was inserted into the lower abdominal aorta via the left femoral artery for BP measurement, and another catheter was placed into the left femoral vein for intravenous injection. The catheters were exteriorized through the interscapular skin. After the procedure, each animal was treated intramuscularly with a dose of sodium benzylpenicillin (6 × 10\(^4\) international units) and placed separately in a cage with controlled temperature (23–25 °C) and with free access to food and tap water. After a 2-day recovery period, the animals were placed in individual cylindrical cages containing food and water for BP recording. The aortic catheter was connected to a BP transducer via a rotating swivel that allowed the animals to move freely in the cage. After approx. 14-h habituation, the BP signal was digitized by a microcomputer. SBP, DBP, MAP and HP values from every heartbeat were determined online. The mean and S.D. values of these parameters during a period of 24 h were calculated for each rat. The S.D. of all values obtained during 24 h was denoted as the quantitative parameter of variability, i.e. SBPV (SBP variability), DBPV (DBP variability), MAPV (MAP variability) and HPV (HP variability) for each rat.

**BRS measurement**

To determine the function of arterial baroreflex in conscious rats, the methods widely used are derived from that of Smyth et al. [11] first applied on humans. The principle of this method is to measure the prolongation of HP in response to an elevation of BP. With some modifications, this method was used in conscious rats [12,13]. A bolus injection of phenylephrine (5 μg/ml) was used to induce an elevation in BP. The time period of drug injection was approx. 1 s. The dose of phenylephrine (2–5 μg/kg of body weight) was adjusted to raise SBP between 20 and 40 mmHg. BRS was calculated over the time the BP was rising. HP was plotted against SBP for linear regression analysis and the slope of SBP–HP was expressed as BRS (ms/mmHg). As there exists a delay (approx. 1 s) between the stimulus and response, the slopes were calculated by computer with 1–10 beats of shift for linear regression analysis and the slope with the highest correlation coefficient was used as BRS. A correlation analysis with five beats of shift, for example, means that values of HP\(_6\)/SBP\(_1\), HP\(_7\)/SBP\(_2\), HP\(_8\)/SBP\(_3\), etc. were used.

**Morphological examination**

Morphological examinations were performed after BP recording and BRS studies. The animals were weighed and killed by decapitation. The thoracic and peritoneal cavities were opened immediately. The right kidney, aorta and heart were excised and rinsed in ice-cold physiological saline. The right kidney was blotted. The left ventricle was isolated, blotted and weighed. At the same time, the aorta was cleaned of adhering fat and connective tissue. Just below the branch of the left subclavicular artery, a 30-mm-long segment of thoracic aorta was harvested, blotted, and weighed. Ratios of LVW/BW (left ventricular weight to body weight) and AW/length (aortic weight to the length of aorta) were calculated [14,15]. Histopathological observation was also carried out with our conventional method [16]. Briefly, immediately after gross detection, all samples of kidneys were immersed in formalin solution for more than 1 week, dehydrated in ethanol, cleared in dimethylbenzene and embedded in paraffin. The sections (5 μm thick) were then prepared and stained with haematoxylin and eosin for light microscopic evaluation.

**GSS (glomerulosclerosis score)**

For the semi-quantitative evaluation of glomerular damage, GSS was defined as described previously [17]. On the light microscopic specimens, approx. 50 glomeruli
from the outer cortex and the same number of glomeruli from the inner cortex for each kidney were graded according to the degree of sclerosis: 0, if no mesangial expansion; 1, if mild mesangial expansion (<30% of a glomerular area); 2, if moderate mesangial expansion (30–60% of a glomerular area); 3, if marked mesangial expansion (>60% of a glomerular area); and 4, if the sclerosis was global. This was performed by one observer in a blinded fashion using coded slides. A weighted composite sclerosis score was then calculated for each kidney according to the following formula: 

$$GSS = \left[ \frac{1 \times (\text{number of grade 1 glomeruli}) + 2 \times (\text{number of grade 2 glomeruli}) + 3 \times (\text{number of grade 3 glomeruli}) + 4 \times (\text{number of grade 4 glomeruli})}{\text{number of glomeruli observed}} \right] \times 100$$

**Statistical analysis**

Data are expressed as means ± S.D. Comparisons between two groups were made by Student’s t test. The relationships between haemodynamic parameters and organ damage parameters were analysed by classic univariate correlation analysis. Stepwise multiple regression analysis was performed to study the independent effect of haemodynamic parameters on organ damage. F to enter and F to remove were set to P < 0.05 and P > 0.10 respectively. P < 0.05 was considered statistically significant. Statistical analysis was performed by using software SPSS 11.0.0.

**RESULTS**

**Effects of low-dose ketanserin on BP, BPV and BRS in SHR**

As shown in Table 1, long-term treatment with ketanserin (0.1 mg·kg⁻¹·day⁻¹) had no obvious effect on SBP, DBP and MAP, but significantly decreased SBPV (−24%; P < 0.01), DBPV (−22%; P < 0.05) and MAPV (−20%; P < 0.05) and obviously enhanced BRS (+92%; P < 0.01) in SHR. In the treated and untreated SHR, BRS was not correlated with SBP (r = −0.156, P > 0.05), DBP (r = −0.082, P > 0.05) or MAP (r = −0.117, P > 0.05). No obvious change was found in HP and HPV.

**Effects of low-dose ketanserin on end-organ damage in SHR**

There was no difference in body weight between ketanserin-treated and untreated SHR. Among organ damage parameters studied, LVW/BW, AW/length and GSS are shown in Table 2. It was found that long-term treatment with ketanserin (0.1 mg·kg⁻¹·day⁻¹) significantly decreased LVW/BW (−11%; P < 0.05) and GSS (−15%; P < 0.05) in SHR. AW/length was decreased slightly (−8%), but this did not reach statistical significance.

**Relationships between BP, BPV, BRS and organ damage in SHR**

Relationships between BP, BPV, HP, HPV, BRS and organ damage in treated and untreated SHR by univariate correlation analysis are shown in Table 3. It was found that LVW/BW was positively correlated with SBPV and negatively correlated with BRS. AW/length was positively correlated with SBP, MAP, SBPV, MAPV and DBPV. GSS was positively correlated with SBPV, MAPV and DBPV, and negatively correlated with BRS. In addition, neither LVW/BW nor GSS were significantly correlated with SBP, DBP and MAP.

The relative dependencies of organ damage on haemodynamic parameters were assessed by stepwise multiple regression analysis. LVW/BW was independently associated with lower BRS (β = −0.522, P < 0.05; where β is the standardized partial regressive coefficient). AW/length was independently associated with higher DBPV (β = 0.604, P < 0.01). GSS was independently associated
Table 3  Linear regression coefficient ($r$) between BP, BPV, HP, HPV and BRS values and organ damage in treated and untreated SHRs

<table>
<thead>
<tr>
<th></th>
<th>LVW/BW</th>
<th>AW/length</th>
<th>GSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>0.294</td>
<td>0.476*</td>
<td>0.307</td>
</tr>
<tr>
<td>DBP</td>
<td>0.162</td>
<td>0.379</td>
<td>0.339</td>
</tr>
<tr>
<td>MAP</td>
<td>0.220</td>
<td>0.421*</td>
<td>0.324</td>
</tr>
<tr>
<td>HP</td>
<td>−0.116</td>
<td>0.012</td>
<td>0.226</td>
</tr>
<tr>
<td>SBPV</td>
<td>0.458*</td>
<td>0.424*</td>
<td>0.522*</td>
</tr>
<tr>
<td>DBPV</td>
<td>0.304</td>
<td>0.604**</td>
<td>0.570**</td>
</tr>
<tr>
<td>MAPV</td>
<td>0.403</td>
<td>0.501**</td>
<td>0.537**</td>
</tr>
<tr>
<td>HPV</td>
<td>0.239</td>
<td>0.101</td>
<td>0.390</td>
</tr>
<tr>
<td>BRS</td>
<td>−0.522*</td>
<td>−0.301</td>
<td>−0.665**</td>
</tr>
</tbody>
</table>

$P < 0.05$ and $** P < 0.01$. $n = 23$.

Figure 1  Correlations between haemodynamic and organ-damage parameters in treated and untreated SHR $n = 23$.

with lower BRS ($\beta = −0.542, P < 0.01$) and higher DBPV ($\beta = 0.405, P < 0.05$). After comparing $\beta$, it was found that the contribution of BRS to renal damage was greater than that of DBPV. Examples of correlations are shown in Figure 1.

DISCUSSION

The present study has directly demonstrated for the first time that ketanserin had an organ-protective effect independent of BP reduction, and this organ protection was importantly attributable to the decrease in BPV and the enhancement of BRS.

In hypertension, impairment of baroreflex is mainly the result of elevated BP levels [18–20]. BRS is enhanced when the BP level is lowered by an antihypertensive drug. However, the present work showed that long-term treatment with low-dose ketanserin (0.1 mg · kg$^{-1}$ · day$^{-1}$) significantly enhanced BRS, but had no obvious effect on BP levels in SHR. These results indicate that this enhancement of BRS was not attributable to the normalization of BP levels. This is in good accordance with our previous study on SHR given ketanserin acutely and on rats with myocardial infarction given chronic ketanserin [8]. In these previous studies [8], it was found that ketanserin (a blocker of 5-HT$_{2A}$ and $\alpha_1$ receptors) increased BRS in SHR when administered either intravenously or intracerebroventricularly; however, prazosin, an $\alpha_1$ receptor blocker, had no obvious effect on BRS. Furthermore, ritanserin (a 5-HT$_{2A}$ receptor blocker) enhanced BRS only following intracerebroventricular administration. This drug does not readily penetrate the blood–brain barrier when administered intravenously [3,8]. Accordingly, the effect of ketanserin on BRS that is independent of BP-lowering action may be mainly mediated by a central 5-HT$_{2A}$ receptor. A newly completed study in our laboratory suggests that the action site of ketanserin on the baroreflex arc was within the ventrolateral medulla in anaesthetized rats (Y. J. Fu, W. Z. Wang and D.-F. Su, unpublished work).

In the present study, SBPV, DBPV and MAPV in SHR were all significantly reduced by long-term treatment with low-dose ketanserin. It is well known that the main function of the baroreflex is to maintain the stability of BP. Therefore the enhancement of impaired baroreflex by the drug may contribute to BPV reduction.

The present work also clearly demonstrated that long-term treatment with low-dose ketanserin produced obvious organ protection in SHR. It is well known that a high BP level induces organ damage and that decreasing BP level can prevent organ damage. However, the present study has shown that, with the dose tested, ketanserin had no significant effect on BP level. This result indicated that ketanserin has an organ-protective action that is independent of its BP-lowering action in SHR.

Clinical observations have suggested that organ damage is related to BPV in hypertensive patients [1,21,22,24]. Moreover, in animal studies, it has been reported that the organ damage induced by sinoaortic denervation was related to the high BPV, but not to the BP level [9,25]. Accordingly, it seems very important to emphasize the role of BPV in antihypertensive therapy [26]. In the present study, univariate correlation analysis showed that LVW/BW, AW/length and GSS were all positively correlated with BPV. These results indicate that a decrease...
in BPV might be one of the major mechanisms for the organ protection of low-dose ketanserin in SHR. It should be noted that, in the present study, the contribution of DBPV was greater than that of SBPV and MAPV to aortic hypertrophy (expressed as AW/length) and renal damage (expressed as GSS). Only DBPV, but not SBPV or MAPV, was significantly related to organ damage in multiple-regression analysis. This surprising result indicates that we should not ignore the importance of DBPV in studies of BPV.

Arterial baroreflex dysfunction is another feature of hypertension. It has been well recognized that BRS is impaired in hypertensive humans and animals [3,4,27]. Our previous study indicated that BRS is one of the independent variables that is related to end-organ damage in hypertension [28]. In the present study, multiple regression analysis showed that amelioration of renal lesion and decrease in left ventricular hypertrophy were all independently associated with the increase in BRS. Therefore enhancement of baroreflex function may importantly contribute to the organ protection of low-dose ketanserin in SHR; however, the exact mechanisms underlying this effect are still unclear. There are two possible explanations. (i) Reduction of BPV. An enhanced baroreflex function may reduce BPV. BPV reduction may contribute to organ protection as mentioned above. (ii) Inhibition of inflammation. It was found that impaired baroreflex function can initiate an inflammatory reaction as indicated by an increase in plasma TNFα (tumour necrosis factor α) and IL-1β (interleukin-1β) in sinoaortic denervated rats [29]: it is well known that inflammation can lead to organ damage.

In conclusion, low-dose ketanserin produces organ protection independently of its BP-lowering action in SHR, and this organ protection was importantly attributable to the enhancement of BRS and the reduction of BPV.

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REFERENCES

23 Reference deleted

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