

Research article

## Omapatrilat normalizes renal function curve in spontaneously hypertensive rats

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### Abstract

**Background:** The present study was designed to analyze the chronic renal response to omapatrilat, a new vasopeptidase inhibitor, in spontaneously hypertensive rats (SHR). To that end, the renal and blood pressure response to a 4-day salt loading protocol was analyzed and the respective chronic renal curves constructed.

**Results:** In non treated animals, and under normal sodium intake (around 2 mEq/day), mean arterial pressure (MAP), was significantly higher in the SHR as compared with the controls (WKY). After increasing salt intake (8 times normal), MAP did not change significantly in any group and the animals reached a normal sodium balance in four days. In a second group of animals, omapatrilat was given orally for 15 days at the dose of 40 mg/kg/day in the drinking water. In these omapatrilat-treated animals, and under normal sodium intake, MAP was significantly lower in both groups, although the antihypertensive effect was much greater in the SHR, so that the MAP of the SHR group was completely normalized and similar to the WKY-treated group. The subsequent elevation of sodium intake did not significantly elevate MAP in any group and the animals could manage the sodium excess as well as the non treated groups.

**Conclusions:** These results indicate that chronic treatment with omapatrilat normalizes blood pressure in SHR without affecting adversely the renal ability to eliminate a sodium load. Chronic treatment with omapatrilat resets the chronic pressure natriuresis relationship of the SHR to a normal level, thus without altering the normal salt-independence of this arterial hypertension model.

### Background

Vasopeptidase inhibitors, agents that inhibit both neutral endopeptidase and angiotensin-converting enzyme, have recently emerged as a novel pharmacological tool for the treatment of cardiovascular and renal diseases. Omapatrilat, one of this new inhibitors, has been shown

to be very effective as an antihypertensive agent. In fact, different laboratories have shown that the omapatrilat antihypertensive effects are greater than those obtained with other commonly used antihypertensive drugs, such as angiotensin converting enzyme inhibitors (ACEI) [1].

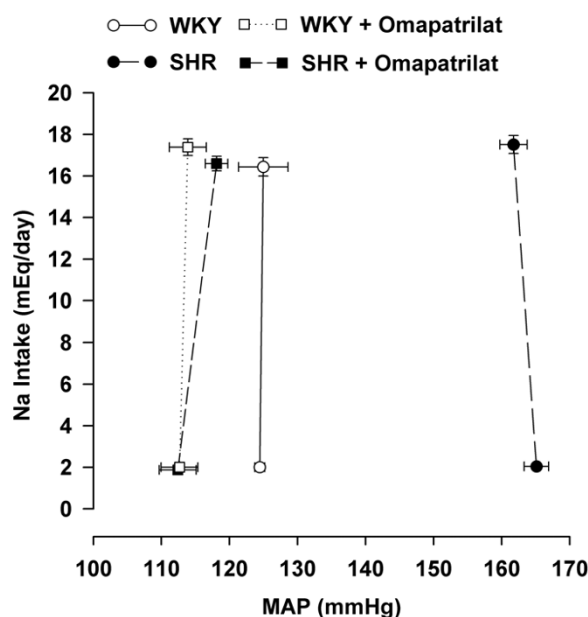
It is known that the mechanism of action of omapatrilat is double. On one hand, it reduces the formation of angiotensin II. On the other hand, it allows the accumulation of natriuretic factors, such as bradykinin. Thus, omapatrilat affects both the vascular tone and renal function, and this has been documented in several forms of experimental hypertension [2-4]. However, the characterization of an antihypertensive drug such as omapatrilat can not be considered complete unless the pressure natriuresis relationship is determined. It is known that a resetting of the pressure natriuresis mechanism is involved in the antihypertensive effect of many drugs [5]. Thus, angiotensin converting enzyme inhibitors reduce blood pressure but also the slope of the pressure natriuresis relationship, thus making blood pressure sensitive to salt intake [6,7]. The presence of an additional direct natriuretic effect in the omapatrilat molecule may change the pressure natriuresis relationship in a different way as with ACEIs. Thus, in the present study we have examined the chronic pressure natriuresis relationship during the administration of omapatrilat in the SHR model of experimental hypertension.

## Results

In the non treated animals and under normal sodium intake, mean arterial pressure (MAP, figure 1), was significantly higher in the SHR ( $165.1 \pm 1.8$  mmHg) as compared with the controls (WKY,  $124.5 \pm 2.8$ ). After increasing salt intake (8 times normal), MAP did not change significantly in any group ( $161.8 \pm 2.0$  and  $124.9 \pm 3.6$ , respectively). There were no significant differences in sodium balance while the animals were on a normal sodium intake. After elevating sodium intake, these animals reached a normal sodium balance in four days (figure 2).

In a second group of animals, omapatrilat was given orally for 15 days at the dose of 40 mg/kg/day in the drinking water. In these omapatrilat-treated animals, and under normal sodium intake, MAP was significantly lower in both groups ( $112.4 \pm 2.7$  and  $112.7 \pm 2.7$ , in SHR and WKY respectively), although the antihypertensive effect was much greater in the SHR, so that the MAP of the SHR group was completely normalized and similar to the WKY-treated group. The subsequent elevation of sodium intake did not significantly elevate MAP in any group ( $118.1 \pm 1.7$  vs  $113.9 \pm 2.7$ , respectively, figure 1). These omapatrilat-treated animals could also manage the sodium excess as well as the non treated groups (figure 2).

There were no important changes in renal glomerular filtration rate (GFR). Under normal sodium intake, the GFR of the WKY group was  $1.7 \pm 0.1$  ml/min/gkw and  $1.6 \pm 0.1$  in the SHR. The elevation of sodium did not change significantly GFR in any group. Pretreatment with oma-



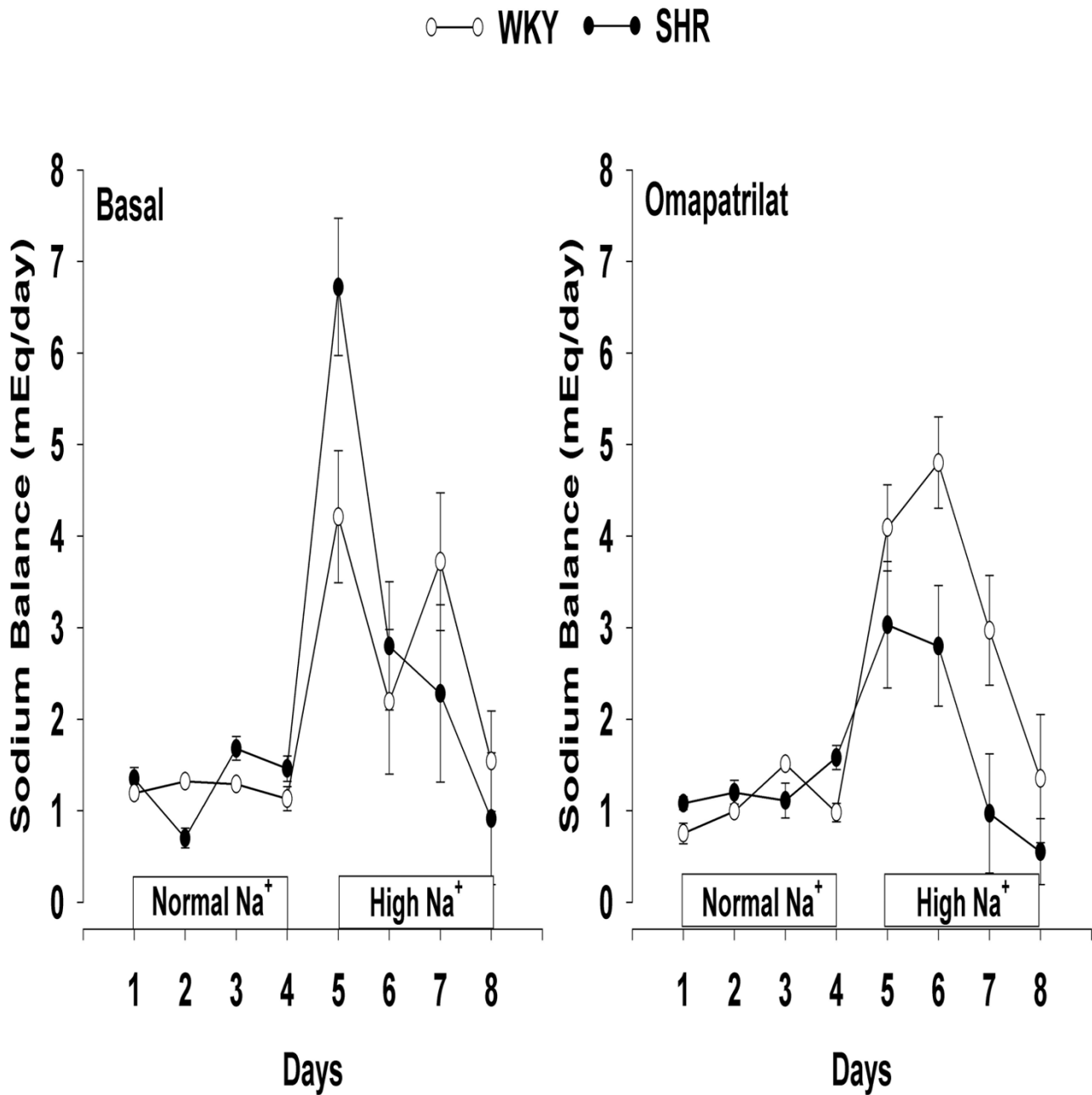
**Figure 1**

Chronic renal function curves in SHR and WKY rats before and during chronic treatment with omapatrilat. MAP, mean arterial pressure.

patrilat did not produce statistical differences between groups ( $1.5 \pm 0.1$  in WKY and  $1.7 \pm 0.1$  in the SHR) under the normal sodium intake and the increase of sodium intake also was without an effect.

## Discussion

In the present study we have characterized the antihypertensive effect of omapatrilat in the spontaneous arterial hypertensive rat model by analyzing the renal and blood response response to an elevation of salt intake. The characterization of the chronic pressure natriuresis relationship allows a precise estimation of the kidney-fluid mechanism that controls arterial pressure, as stated by Guyton and coworkers in a very elegant series of experiments [8]. As opposed to the acute pressure natriuresis relationship, which has to be performed in anesthetized animals by changing the renal perfusion pressure [9,10], the chronic pressure natriuresis relationship characterized in the present report is obtained in undisturbed animals by changing the intake of salt and water. In any case, however, both approaches should give the same estimate of the so-called equilibrium point at which blood pressure is set for any intake of sodium and water. This equilibrium point, also called set-point, is therefore the blood pressure that will be reached in the long-term, in any physiological or pathological situation [5,6].



**Figure 2**  
Daily sodium balance in SHR and WKY rats before and during chronic treatment with omapatrilat. \*, p < 0.05 vs groups.

The results contained in this manuscript clearly show, as in other previous studies, that omapatrilat is a very potent antihypertensive effect. In fact, it has been reported that omapatrilat appears to have even greater antihypertensive effects in SHR than ACEIs alone [1]. In our hands, blood pressure was normalized by the chronic treatment with omapatrilat, while in other studies using only an ACEI such as enalaprilat, blood pressure re-

mained slightly above the normal values [11]. The mechanisms behind this beneficial effect of omapatrilat may be double. Both the decrease in the formation of angiotensin II and the accumulation of natriuretic peptides should, at least theoretically, contribute to the decrease in blood pressure, through both a vasodilatory action and the elimination of more salt and water.

Although the blood pressure lowering effect of vaso-peptidase inhibitors has been shown by many different laboratories, its effects on renal function have not been characterized. Our results clearly demonstrate that omapatrilat does not negatively affect the renal function of the hypertensive animals. Thus, glomerular filtration rate was not adversely affected and also the ability to eliminate a salt excess was also very well preserved, in a similar way as the untreated normotensive rats. Thus, the slope of the chronic pressure natriuresis curve was not depressed, neither by the decrease in blood pressure nor by the drug itself, and the steepness of this relationship was maintained at the same level as the controls. Previous studies using converting enzyme inhibitors in normal animals have shown that they effectively decrease blood pressure, even to hypotensive levels, but the slope of the chronic pressure natriuresis relationship is somewhat depressed, thus making blood pressure salt-sensitive [5–8]. In the present experiments, however, the chronic pressure natriuresis curve of the normotensive animals treated with omapatrilat was displaced to the left, that is to lower pressures, but the slope was not significantly changed, remaining essentially the same. Thus, the normotensive animals were not salt-sensitive before the administration of the drug and they also remained salt-resistant after being treated with the vaso-peptidase inhibitor. The same can be safely said for the hypertensive animals. It is likely that the difference between ACEIs alone and omapatrilat may be related to the greater natriuretic potency of the latter. In fact, the omapatrilat-treated SHR showed a greater ability to excrete the sodium load, as evidenced by the lower sodium retention shown at day 1 (figure 2). Moreover, recent data suggest that omapatrilat treatment is superior to captopril in improving the altered endothelial function of a model of salt-sensitive hypertension [12,13]. It is also interesting to add that some authors have shown that SHRs, or at least some substrains of SHR, can be identified as salt-sensitive [14], in a similar way as other classical salt-sensitive rat models such as the DOCA-salt or the Dahl-sensitive rats. However, the animals used in the present study did not show this tendency to increase their blood pressure in response to salt loading.

Thus, it can be concluded that chronic treatment with omapatrilat decreases blood pressure in SHRs, resetting the pressure natriuresis relationship to a normal level, thus without altering the normal salt-independence of this arterial hypertension model.

### Material and Methods

Male spontaneously hypertensive rats (SHR) and their normotensive controls (WKY), born and raised in the Animal House of the Universidad de Murcia, were used for the study (16 weeks of age). The animals were main-

tained on standard rat chow and tap water throughout the study, unless otherwise stated. All experiments were performed according to the guidelines of the European Union for the ethical treatment of the animals.

### Experimental groups and protocol

Four groups of 8 animals each were used in the present study. One group of WKY and SHR rats served as the untreated controls and one more group of WKY and SHR were treated chronically for 15 days with omapatrilat (40 mg/kg/day in the drinking water).

To construct the renal function curves, the animals were housed in metabolic cages and after 2 days of getting used to the cages, sodium balance (mEq/day) was calculated daily by subtracting sodium intake [food intake (g/day)  $\times$  0.104 mEq/g (sodium content of the food)] and sodium excretion [diuresis (ml/day)  $\times$  urine sodium concentration (mEq/l)]. Two periods of four days each were observed. In the first one (normal sodium intake), the animals were offered normal pulverized rat chow and tap water. In the second one (high sodium intake), started immediately after the normal sodium period, sodium intake was elevated 8 times by adding the appropriate amounts of NaCl to the rat chow. Blood pressure was measured twice in each period, before the period started and after the period finished. In the case of the groups treated with omapatrilat, the animals were housed in the metabolic cages only the last 8 days of the treatment, to obtain the normal salt and the salt loading responses.

Blood pressure was determined by the tail cuff method (Cibertec, Madrid, Spain) by using MacLab software (AD Instruments, UK) in a Macintosh LCII computer. This approach allowed the estimation of systolic and diastolic blood pressures, and then of mean arterial pressure. Urine samples were measured by gravimetry, centrifuged and frozen to measure urinary creatinine and sodium concentrations. A small blood sample was also obtained from the tail at the moment of blood pressure measurement for measurement of plasma creatinine.

### Analytical techniques

Glomerular filtration rate was estimated as the clearance of creatinine (urine to plasma concentration ratio times diuresis). Plasma and urine creatinine were measured by the Jaffe reaction using a commercially available kit (Boehringer Mannheim, Barcelona, Spain). Sodium concentration was measured by an ion selective electrode.

### Statistical Methods

Data are presented as means  $\pm$  S.E.M. A repeated measures analysis of variance was used to obtain the statistical significance between and within groups. Differences

were considered statistically significant at a P level lower than 0.05.

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