Role of Substance P in Blood Pressure Regulation in Salt-Dependent Experimental Hypertension

Osvaldo Kohlmann, Jr, Mario Luís Cesaretti, Milton Guoza, Agostinho Tavares, Maria Teresa Zanella, Artur Beltrame Ribeiro, Oswaldo Luiz Ramos, Susan E. Leeman, Irene Gavras, Haralambos Gavras

Abstract The participation of substance P in the pathogenesis of five models of experimental hypertension, i.e., DOCA-salt, subtotal nephrectomy, one-kidney–one clip renovascular, two-kidney–one clip renovascular, and spontaneous hypertension, was evaluated via an acute infusion of a newly synthesized potent, specific nonpeptide antagonist of substance P at the NK-1 receptor, the agent CP 96,345. In conscious unrestrained rats, CP 96,345 induced significant and sustained increases in mean arterial pressure of DOCA-salt, subtotal nephrectomy, and one-kidney–one clip renovascular hypertensive rats but only small and nonsignificant changes in blood pressure of two-kidney–one clip renovascular and spontaneously hypertensive rats CP 96,345 had no effect on the blood pressure of sham-treated controls and Wistar-Kyoto rats. This NK-1 receptor antagonist did not significantly affect the heart rate of any experimental model studied. The data suggest that endogenous substance P may act as a partial counterregulatory mechanism against vasoconstriction in models of salt-dependent hypertension (Hypertension. 1997;29[part 2]:506-509).

Key Words • experimental hypertension • sodium-dependent hypertension • substance P • receptors NK-1 • tachykinins

Substance P, a member of the family of tachykinins, is involved in numerous physiological activities, such as neurotransmission–neuromodulation, stimulation of salivary secretion, smooth muscle contraction, and vasodilation.

The vasodilatory action of substance P is endothelium dependent and is mediated by the neurokinin NK-1 receptors located on the endothelial cells. Besides its vasodilatory properties, in some vascular beds substance P also exerts a contracting action on the vascular smooth muscle. These vasoactive properties of substance P enable this peptide to participate in the regulation of blood flow of various organs.

The participation of endogenous substance P in hypertension has been evaluated by a few investigators, mainly by measurement of the plasma levels of this peptide, whereas the pharmacological effects have been explored by administration of synthetic substance P or related analogues. Reduced plasma levels of this peptide have been described in both stroke-prone spontaneously hypertensive rats and in human essential hypertension, suggesting that elevated blood pressure in these subjects could be related, at least in part, to the lack of adequate counterregulatory action exerted by this endogenous vasodilator.

Recently, a potent, specific, nonpeptide antagonist of the NK-1 receptor, devoid of agonistic actions was synthesized: the compound CP 96,345. This has allowed more precise and systematic evaluation of the physiological properties of endogenous substance P and its participation in hypertension.

In previous studies from our laboratories, we were able to demonstrate that an acute infusion of this compound increases blood pressure of DOCA-salt hypertensive rats in the early stages (6 weeks) of the hypertensive state. However, when we infused the same agent in rats at later phases of DOCA-salt hypertension (12 weeks), no significant increases in blood pressure were observed. These observations suggest that participation of substance P in the pathogenesis of certain types of hypertension may vary in different stages of the disease depending on the mechanisms prevailing at each stage.

The aim of this study was to extend our previous observations to other models of experimental hypertension with different pathogenetic mechanisms by determining the blood pressure and heart rate effects of an acute administration of the compound CP 96,345.

Methods

Animals

Male Wistar, Wistar-Kyoto (WKY), and spontaneously hypertensive rats (SHR) were used in this study. Animals weights varied in accordance to the model of hypertension. Animals were housed in cages containing five rats each. They were maintained in a room with constant temperature, 12-hour light/dark cycles, and with free access to regular rat chow and tap water unless otherwise specified. All animals were obtained from the Animal Care Center and Breeding Laboratories of Federal University of São Paulo, Brazil. SHR and WKY colonies are derived from the National Institute of Health and are cataloged in the International Index of Laboratory Animals.

The experimental protocol for this study was approved by the Animal Care and Use Committee of Federal University of São Paulo and was conducted according to the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health.
Five experimental hypertensive models and their appropriate controls were used in this study. They were prepared as follows:

1. **DOCA-salt hypertension** was induced in nine uninephrectomized Wistar rats weighing 290 to 320 g by weekly subcutaneous injections of deoxycorticosterone pivalate 20 mg/kg body weight and 1% saline to drink instead of tap water. Control sham-treated rats (n=6) were also uninephrectomized but were injected weekly with distilled water and drank tap water instead of 1% saline. All were studied at the sixth week of hypertension.

2. **Subtotal nephrectomy hypertension** was induced in six Wistar rats weighing 300 to 340 g by ligating the upper and lower pole arteries of the left kidney and excising the right kidney. They received 1% saline to drink ad libitum. Six uninephrectomized sham-operated animals, drinking tap water instead of 1% saline, were used as controls. Animals were studied at 6 weeks after the surgical procedures.

3. **One-kidney–one-clp renovascular hypertension (1K-1C)** Six male Wistar rats weighing 180 to 200 g had a silver clip with a gap size of 0.20 mm placed around the left renal artery and had the right kidney removed. Uninephrectomized sham-operated animals (n=5) were prepared as controls. Six weeks after clipping the renal artery, they received the NK-1 receptor antagonist.

4. **Two-kidney–one-clp renovascular hypertension (2K-1C)** In six male Wistar rats weighing 180 to 210 g, a silver clip with a gap size of 0.20 mm was placed around the left renal artery. The right kidney was left intact. Controls for this group were seven sham-operated rats. They were studied 8 weeks after induction of hypertension.

5. **SHR** SHR at two different ages were studied, young (6 to 8 weeks of age, weight 180 to 200 g, n=7) and adult (12 to 16 weeks of age; weight 270 to 310 g, n=7). Normotensive age-matched WKY (n=5) were used as controls.

### Antagonism of the (NK-1) Receptor of Substance P

The compound CP 96,345 ([2(S),3S]-cis-[diphenylmethyl]-N-[2-methoxyphenyl]-methyl)-1-azabicyclo[2,2,2]octan-3-amine), a potent and specific nonpeptide antagonist of the NK-1 receptor,\(^{1,16}\) was diluted in distilled water and infused intravenously at a rate of 30 μg kg⁻¹ min⁻¹ (12 μL/min) for 60 consecutive minutes. This dosage was chosen on the basis of previous experiments from our laboratories.\(^{1,10}\)

In unanesthetized, unrestrained animals housed in individual cages, mean arterial pressure (MAP) and heart rate (HR) were recorded directly in a polygraph (Gould Inc) before and every 5 minutes during the 60-minute period of CP 96,345 intravenous infusion. Each recorded value represents the mean of five readings obtained during 1 minute of continuous BP monitoring. For these experiments, animals had indwelling catheters placed in the femoral artery (for MAP measurements) and femoral vein (for drug infusion) under light ether anesthesia on the day before the study. The catheters were tunneled subcutaneously and exteriorized at the nape of the animal.

As previously published,\(^{1,9}\) the vehicle infused alone at this rate had no effect on BP of DOCA-salt hypertensive rats, and that was also confirmed in this study in some animals from DOCA-salt and subtotal nephrectomy groups.

Results are presented as mean±SD. Two-way ANOVA complemented with Mann-Whitney test with a significance level of 5% was used for comparison of BP and HR values before and during the infusion of the antagonist among groups.

### Results

Fig 1 shows that administration of the NK-1 receptor antagonist of substance P to subtotally nephrectomized rats produced significant and sustained increases in MAP starting at 10 minutes from the beginning of the infusion and lasting for the duration of the infusion period (P<0.01) in sham operated rats. It caused only small, transient, and nonsignificant increases in BP.

A similar pattern and magnitude of BP increase during CP 96,345 infusion was observed for DOCA-salt hypertensive rats. BP of DOCA-salt rats increased from 158±12 mm Hg at the baseline period to an average of 179±10 mm Hg during CP 96,345 infusion, and this increase in BP was sustained throughout the infusion period. Again, for sham-treated animals, no BP effect of this antagonist was observed.

In 1K-1C renovascular hypertensive rats, significant increases in MAP were also observed during infusion of the NK-1 receptor antagonist. The average MAP increase in this group was 15±10 mm Hg, but the BP values remained significantly greater than baseline only up to the 35th minute of the infusion period. In the last 25 minutes of the infusion of CP 96,345, MAP values did not differ from baseline.

Fig 2 summarizes the BP effects of CP 96,345 in these three hypertensive groups and in sham animals pooled together by showing the mean values for BP before and during the infusion of this compound. Mean increases in BP were 24±1.1 and 21±0.9 mm Hg for the subtotally nephrectomized and DOCA-salt groups, respectively (P<0.01) in the 1K-1C renovascular model. MAP went up from 168±16 mm Hg (before) to 183±14 mm Hg during CP 96345 infusion (P<0.05). For the sham-operated controls, mean BP during the antagonist infusion was not different from baseline.

Infusion of the NK-1 receptor antagonist did not cause a significant change in MAP of animals from either the 2K-1C renovascular hypertensive group or its sham-operated control group, as shown in Fig 3.

Also, BP of both young and adult SHR was not significantly altered by CP 96,345, nor was that of their normotensive control group of WKY (Fig 4).

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**Selected Abbreviations and Acronyms**

- 1K-1C = one-kidney, one clip
- 2K-1C = two-kidney, one clip
- BP = blood pressure
- HR = heart rate
- MAP = mean arterial pressure
- SHR = spontaneously hypertensive rats
- WKY = Wistar-Kyoto rats

**Hypertension Models**

FIG 1 MAP (mm Hg) in subtotally nephrectomized (A) or sham-operated control group, as shown in Fig 3.

FIG 2 Summary of the BP effects of CP 96,345 in these three hypertensive groups and in sham animals pooled together by showing the mean values for BP before and during the infusion of this compound. Mean increases in BP were 24±1.1 and 21±0.9 mm Hg for the subtotally nephrectomized and DOCA-salt groups, respectively (P<0.01) in the 1K-1C renovascular model. MAP went up from 168±16 mm Hg (before) to 183±14 mm Hg during CP 96345 infusion (P<0.05). For the sham-operated controls, mean BP during the antagonist infusion was not different from baseline.
No significant changes in HR were observed throughout the administration of the NK-1 receptor antagonist in any hypertensive or control group (Table).

**Discussion**

Our results confirm previous data from our laboratories demonstrating that in the early phases of DOCA-salt hypertension, acute inhibition of substance P by a potent and specific nonpeptide antagonist of the NK-1 receptor causes sustained increases in BP. The current studies extend these observations to another two salt-dependent experimental models of hypertension subtotal nephrectomy and 1K-1C renovascular hypertension. However, in the 1K-1C model, the magnitude of BP elevation and duration of the effect were somewhat less than those observed in subtotal nephrectomized and DOCA-salt rats.

On the other hand, our data also indicate that this endogenous vasodilator is not always involved in the pathogenesis of elevated BP. Indeed, the acute administration of CP 96,345 given at the same dosage to two other experimental hypertensive models, the 2K-1C renovascular and the SHR, did not cause a significant change in BP. Moreover, in spontaneous hypertension, this NK-1 receptor antagonist had no cardiovascular effects in either the early or late stages of hypertension. These observations are in agreement with previous data from our laboratories demonstrating that this antagonist, when infused at later phases of the DOCA-salt hypertension, does not increase BP, suggesting that the participation of substance P in the pathogenesis of elevated BP could vary according to the phase of the hypertensive state and/or the experimental model.

The BP effect of the antagonist used in the present study was due to blockade of the NK-1 receptor, since in previous studies we demonstrated that the enantiomer of this compound, which has been found to be inactive in vitro, did not affect BP in either DOCA-salt or sham-treated animals. This, of course, does not preclude the possibility of a partial nonspecific effect, e.g., mediated via the action of the compound CP 96,345 on the L-type calcium channels.

Also, as previously shown, the antagonist was infused at a rate that completely abolishes the BP response to exogenous substance P given to normal rats. Therefore, in some experimental models of hypertension, but not in all, the neurotransmitter substance P, which also has endothelium-dependent vasodilatory properties, appears to be acting as a counterregulatory mechanism against vasoconstriction in an attempt to minimize the elevation in BP. These observations would be in keeping with previous data from the literature, in which reduced plasma levels of substance P were observed in experimental and human essential hypertension, suggesting that the elevated BP levels of these entities could be due, at least in part, to insufficient synthesis or release of this endogenous vasodilator.

Why substance P may be involved in some but not all experimental models of hypertension used in the present

**Mean HR Values (bpm) at Baseline and During Infusion of NK-1 Receptor Antagonist in All Experimental Hypertensive Models and in Control Groups**

<table>
<thead>
<tr>
<th>Model</th>
<th>Baseline</th>
<th>NK-1 Receptor Antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOCA-salt</td>
<td>383±52</td>
<td>407±49</td>
</tr>
<tr>
<td>Subtotal nephrectomy</td>
<td>402±32</td>
<td>390±31</td>
</tr>
<tr>
<td>1K-1C</td>
<td>344±33</td>
<td>351±33</td>
</tr>
<tr>
<td>2K-1C</td>
<td>411±46</td>
<td>361±49</td>
</tr>
<tr>
<td>Adult SHR</td>
<td>437±55</td>
<td>390±43</td>
</tr>
<tr>
<td>Young SHR</td>
<td>460±65</td>
<td>428±58</td>
</tr>
<tr>
<td>WKY</td>
<td>305±52</td>
<td>273±27</td>
</tr>
<tr>
<td>Sham-operated control</td>
<td>403±51</td>
<td>363±43</td>
</tr>
</tbody>
</table>
The study remains obscure. The common characteristic among the three experimental models, e.g., DOCA-salt, subtotal nephrectomized, and 1K-1C renovascular, in which the NK-1 receptor antagonist was able to increase BP, is the salt dependency of the hypertensive state. On the other hand, spontaneous hypertension and 2K-1C renovascular hypertension are not salt-dependent models. This also could be an explanation for the previous observations of our laboratories in later phases of DOCA-salt hypertension, since at that stage the salt dependency of the hypertensive state is known to be less important.

The mechanisms by which the sodium dependency could influence the participation of substance P in hypertensive states, e.g., direct effects on synthesis/secretion of this endogenous vasodilator or indirectly, via central sympathetic stimulation, are not known. Our current data do not permit further speculation along these lines.

An alternative explanation for our results that cannot be ruled out is a nonspecific effect of CP 96,345 on L-type calcium channels. However, in such case, a BP-lowering effect ought to have occurred in all hypertensive models, because calcium channel blockers are known to affect all types of hypertension. Further studies are necessary to evaluate these possibilities.

HK was unaffected by CP 96,345 in all models studied, even in those in which large and sustained increases in BP were observed during the infusion of this NK-1 receptor antagonist, pointing to a lack of baroreflex response in these animals. The reasons and mechanisms for the apparent impairment of baroreflex response after NK-1 antagonist infusion are not known, and our data do not allow us to explore them. However, since substance P is an important neurotransmitter widely distributed in the central nervous system, including the baroreflex complex, and CP 96,345 is able to antagonize NK-1 receptors in the central nervous system, an effect of this compound at that level cannot be excluded as an explanation for the lack of an adequate baroreflex response to the acute elevation in BP.

On the basis of our present findings, we propose the hypothesis that in some models of experimental hypertension, substance P could be playing a role as a counterregulatory mechanism against vasoconstriction, in an attempt to attenuate the elevation of BP. This participation appears to be strongly influenced by the sodium dependency of the hypertensive state.

References

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