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# Intracisternal galanin/angiotensin II interactions in central cardiovascular control

Zaida Díaz-Cabiale[a], Concepción Parrado[b], Carmen Vela[a], Rafael Coveñas[c], Noboru Yanaihara[d], Kjell Fuxe[e], Salvador González-Barón[a], José A. Narváez[a,*]

[a]*Departamento de Fisiología, Facultad de Medicina, Campus de Teatinos s/n, Málaga 29080, Spain*
[b]*Departamento de Histologia y Anatomia Patológica, Facultad de Medicina, Campus de Teatinos s/n, Málaga 29080, Spain*
[c]*Instituto de Neurociencias de Castilla y León, Laboratorio de Neuroanatomía de los Sistemas Peptidérgicos, Salamanca, Spain*
[d]*Laboratory of Bio-Organic Chemistry, University of Shizouka, Shizouka, Japan*
[e]*Department of Neuroscience, Karolinska Institute, Stockholm, Sweden*

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**Abstract**

The aim of this work was to investigate the interactions between angiotensin II (Ang II) and galanin(1–29) [GAL(1–29)] or its N-terminal fragment galanin(1–15) [GAL(1–15)] on central cardiovascular control. The involvement of angiotensin type 1 (AT1) receptor subtype was analyzed by the AT1 antagonist, DuP 753. Anesthesized male Sprague–Dawley rats received intracisternal microinjections of Ang II (3 nmol) with GAL(1–29) (3 nmol) or GAL(1–15) (0.1 nmol) alone or in combination. The changes in mean arterial pressure (MAP) and heart rate (HR) recorded from the femoral artery were analyzed. The injection of Ang II and GAL(1–15) alone did not produce any change in MAP. However, coinjections of both Ang II and GAL(1–15) elicited a significant vasopressor response. This response was blocked by DuP 753. Ang II and GAL(1–15) alone produced an increase in HR. The coinjections of Ang II with GAL(1–15) induced an increase in HR not significantly different from the tachycardia produced by each peptide. The presence of DuP 753 counteracted this response. GAL(1–29) alone elicited a transient vasopressor response that disappeared in the presence of Ang II. The coinjections of Ang II with GAL(1–29) and with DuP 753 restored the transient vasopressor effect produced by GAL(1–29). GAL(1–29) produced a slight but significant tachycardic effect that was not modified in the presence of Ang II. The presence of DuP 753 did not modify the tachycardic response produced by Ang II and GAL(1–29). These results give indications for the existence of a differential modulatory effect of Ang II with GAL(1–15) and GAL(1-29) on central blood pressure response that might be dependent on the activity of the angiotensin AT1 receptor subtype.


*Keywords:* Angiotensin; Galanin; Galanin fragment; Cardiovascular control; Rat; Brainstem

## 1. Introduction

Galanin is a 29-amino-acid neuropeptide that was originally isolated from the porcine gut [1]. Both galanin-like immunoreactivity and its specific receptors analyzed by autoradiography and in situ hybridization show a widespread distribution in the central and peripheral nervous systems of mammals [2–6].

Galanin is involved in a variety of functions, such as learning and memory, pain control, food intake, neuroendocrine control, and central cardiovascular regulation [7–10].

With regards to central cardiovascular regulation, intracisternal administration of galanin(1–29) [GAL(1–29)] induces a weak hypotension and tachycardia [10,11] but the N-terminal galanin fragment (1–15) [GAL(1–15)] elicits hypertension and tachycardia and counteracts the cardiovascular actions of the parent molecule, GAL(1–29) [10]. The involvement of the cardiac sympathetic activity in the cardiovascular responses elicited by central administration

* Corresponding author. Tel.: +34 952131646; fax: +34 952131650.
*E-mail address:* bueno@uma.es (J.A. Narváez).

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of the N-terminal galanin fragment (1–15) has been shown since pretreatment with the β-receptor antagonist propranolol blocks the central cardiovascular effects of GAL(1–15) while pretreatment with the cholinergic antagonist atropine is ineffective [12]. Furthermore, GAL(1–15) decreases baroreceptor reflex sensitivity, whereas GAL(1–29) has no effect [13], and the galanin antagonist M40 blocks the central cardiovascular responses elicited by GAL(1–15) without affecting those induced by GAL(1–29) [14]. All these results support a differential role of the N-terminal galanin fragment in central cardiovascular control as compared with the parent molecule, probably mediated by different galanin receptor subtypes.

Also GAL(1–29), but not GAL(1–15), decreases the affinity of the $\alpha_2$-receptor agonist binding sites in the nucleus tractus solitarius (NTS) associated with a significant increase of the $B_{max}$ values [15]. In fact, an antagonistic modulation by GAL(1–29) on $\alpha_2$-adrenoceptors and their functional responses within the NTS has been demonstrated [15]. The quantitative autoradiographical results suggested that this functional antagonistic interaction could take place at the receptor level in the NTS through a galanin receptor/$\alpha_2$ receptor interaction [15]. Thus, it can be suggested that GAL(1–29) receptors, but not the putative GAL(1–15) receptors, interact differentially with other neurotransmitter receptors involved in central cardiovascular function [14,15].

Angiotensin II (Ang II) is a neuropeptide known to be involved in the central cardiovascular control [16]. Ang II-immunoreactive cell bodies and AT1 Ang II receptors have been observed in areas involved in the regulation of blood pressure including the NTS and ventrolateral medulla (VLM) [17–21]. In anesthesized rats, microinjection of Ang II into the NTS produces vasopressor and tachycardic responses at nanomole doses [22–24], but elicits vasodepressor and bradycardic responses at picomole doses [23,25]. Furthermore, microinjections of Ang II into the NTS reduce baroreceptor reflex sensitivity [26], and endogenous Ang II exerts a tonic inhibitory modulation on baroreceptor reflex mediated by AT1 receptors but not AT2 receptors [27]. Moreover, Ang II induces the expression of c-*fos* gene in the NTS via activation of the AT1 receptors [28].

Ang II interacts with neuropeptides and neurotransmitters involved in cardiovascular functions such as substance P (SP), neuropeptide Y, and catecholamines [24,29,30]. It is of special interest that Ang II counteracts the vasodepressor effects of L-adrenaline or the $\alpha_2$-adrenoreceptor agonist clonidine microinjected in the NTS, and increases the $K_d$ and $B_{max}$ values of $\alpha_2$-adrenoreceptor agonist binding [24]. This effect seems to be mediated by AT1 receptor subtype [24]. In this way, Ang II resembles the function of GAL(1–29) but not of GAL(1–15). Thereby, the question that rises is: Can Ang II interact with GAL in cardiovascular functions? Therefore, the aim of this work is to investigate the potential interactions between Ang II and GAL(1–29) and its N-terminal fragment GAL(1–15) on central cardiovascular control by using intracisternal injections. Since Ang II and GAL(1–29) cloned receptors are present not only in the NTS but also in the VLM, the dorsal motor nucleus of the vagus, and the ambiguus nuclei [3,21], we have used intracisternal injections to influence all these cardiovascular regions in the brainstem rather than a specific nucleus. The involvement of the AT1 subtype in this interaction has been analyzed by the use of the AT1 antagonist, DuP 753 [31].

## 2. Materials and methods

### 2.1. Animals

Male-specific pathogen-free Sprague–Dawley rats (body weight: 200–250 g) obtained from Criffa (Barcelona, Spain) were maintained on a regular light–dark cycle (lights on 08:00 h; lights off 20:00 h) in temperature- and humidity-controlled rooms. The animals had free access to food pellets and tap water.

### 2.2. Surgical procedures

The animals were anesthesized with urethane (1.4 g/kg; Sigma, USA) and immediately tracheotomized. The femoral artery was cannulated with a plastic catheter (PE-50; Clay Adams, NY, USA) containing heparin (50 IU/ml of 0.9% NaCl wt/vol) to record blood pressure and heart rate (HR), and the animal was placed in a stereotaxic frame. The head

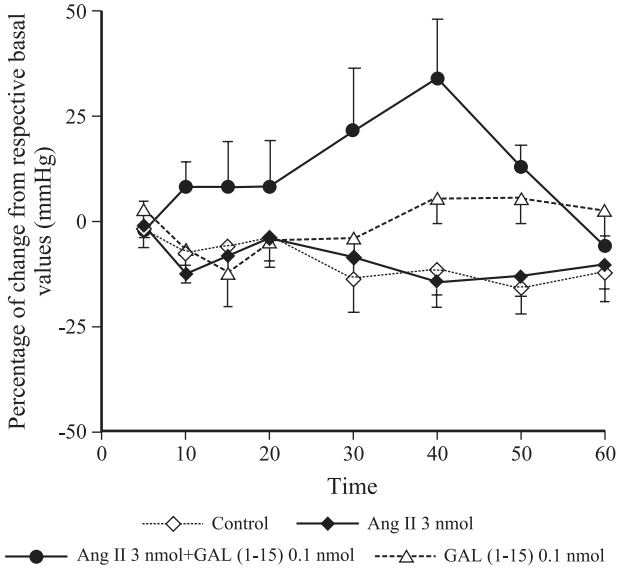


Fig. 1. Effects of intracisternal injections of Ang II (3 nmol/rat), GAL(1–15) (0.1 nmol/rat), GAL(1–15)+Ang II, and CSF on mean arterial blood pressure over a 60-min recording period. Mean±S.E.M. are shown as percentages of changes from the respective basal values. $n$=6–8 rats per group. The basal MAP values were: Ang II group, 84±9 mmHg; GAL(1–15), 81±4 mmHg; GAL(1–15)+Ang II, 75±3 mmHg; CSF group, 74±6 mm Hg. Significances are given in Fig. 2.

was flexed 45°, the neck muscles were dissected with an electric knife to avoid bleeding, and the atlanto-occipital membrane was exposed. The surgical procedure took 8–10 min and the animals were allowed to stabilize for at least 30 min. During the whole experiment, body temperature was maintained at 37.5±0.5 °C by means of a thermostatic blanket.

### 2.3. Intracisternal injections

Intracisternal injections were made stereotaxically through the atlanto-occipital membrane with a Hamilton syringe. Fresh solutions were prepared immediately before the injections by dissolving the peptides in artificial cerebrospinal fluid (aCSF) (composition of the aCSF solution was: 120 mM NaCl, 20 mM $NaH_2CO_3$, 2 mM KCl, 0.5 mM $KH_2PO_4$, 1.2 mM $CaCl_2$, 1.8 mM $MgCl_2$, 0.5 mM $Na_2SO_4$, and 6.8 mM D-glucose). The volume injected was 10 μl in a period of 10–12 s.

### 2.4. Cardiovascular experiments

Mean arterial pressure (MAP) and HR were recorded by connecting the femoral catheter to a Statham-type transducer linked to a computerized data acquisition system (MacLab; AD Instruments).

Table 1
Effect on the heart rate of intracisternal injections of Ang II, GAL(1–29), GAL(1–15), and DuP 753, alone or in combination, on anesthesized rats

| Treatment | Heart rate | | |
|---|---|---|---|
| | Basal value (beats/min) | Decrease | |
| | | Peak (%) | Area (arbitrary units) |
| aCSF | 395±10 | −3.6±1 | −1030±500[a] |
| Ang II [3 nmol] | 388±7 | −4.2±1 | 1249±448 |
| GAL(1–15) [0.1 nmol] | 398±28 | 6.5±1[b,c] | 1438±522 |
| Ang II [3 nmol]+ GAL(1–15) [0.1 nmol] | 414±19 | −6.6±3 | 1790±905 |
| Ang II [3 nmol]+ GAL(1–15) [0.1 nmol] +DuP 753 [0.5 μg] | 408±15 | −6.1±1 | −695±310[a] |
| GAL(1–29) [3 nmol] | 420±13 | 9.7±2[b] | 951±330 |
| Ang II [3 nmol]+ GAL(1–29) [3 nmol] | 408±25 | 8.3±2[b] | 1440±556 |
| Ang II [3 nmol]+ GAL(1–29) [3 nmol]+DuP 753 [0.5 μg] | 416±20 | 6.6±2[b] | 1535±814 |

Mean±S.E.M. are shown ($n$=6–8 rats in each group). The maximal peak effects are expressed as percent change from the respective mean basal value. The area values formed under the curves are expressed in arbitrary units.

[a] $p$<0.01 vs. the rest of the groups according to Kruskall–Wallis followed by Dunn's posttest.

[b] $p$<0.05 vs. aCSF and Ang II.

[c] $p$<0.05 vs. Ang II+Gal(1–15) and Ang II+GAL(1–15)+DuP.

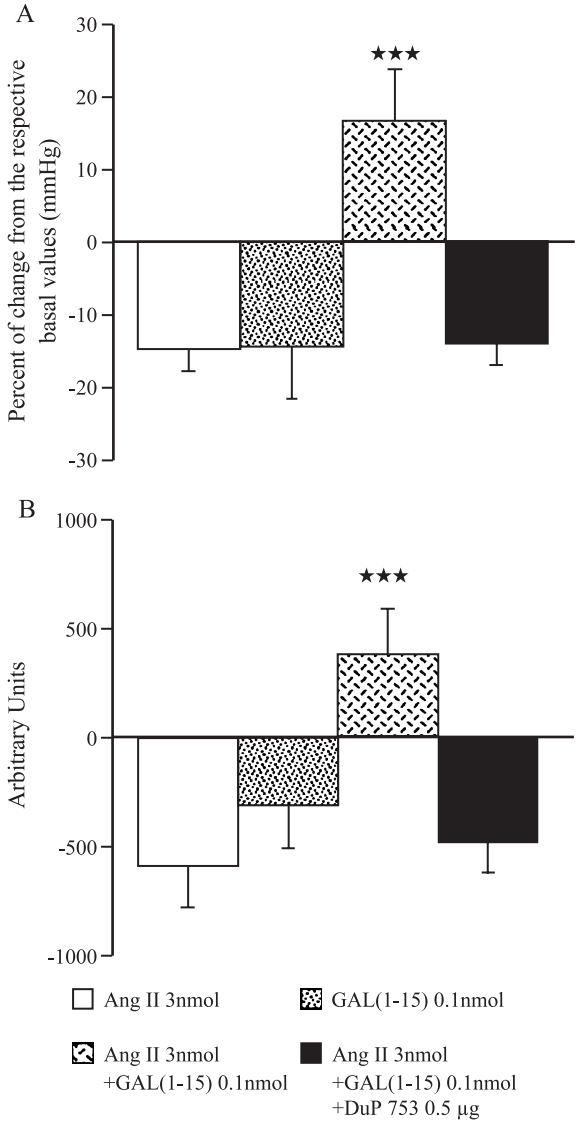


Fig. 2. Effects of intracisternal injections of Ang II (3 nmol/rat), GAL(1–15) (0.1 nmol/rat), GAL(1–15) (0.1 nmol/rat)+Ang II (3 nmol/rat), GAL(1–15) (0.1 nmol/rat)+Ang II (3 nmol/rat)+DuP 753 (0.5 μg), and CSF on mean arterial blood pressure. The peak effect (A) is shown as percent changes from respective basal values and the overall effects (area values) are shown (B) as absolute values in arbitrary units. Mean±S.E.M. are given. $n$=6–8 rats per group. The basal values were described in the legend of Fig. 1. GAL(1–15) (0.1 nmol/rat)+Ang II (3 nmol/rat)+DuP 753 (0.5 μg), 84±5 mm Hg. ***$p$<0.001 vs. the rest of the groups according to Kruskall–Wallis followed by Dunn's posttest.

In order to evaluate the possible role of Ang II with GAL(1–29) or with GAL(1–15) cardiovascular modulation, groups of animals received a threshold dose of Ang II (3 nmol), GAL(1–29) (3 nmol), and GAL(1–15) (0.1 nmol) alone or in combination. Control groups ($n$=6–8) were injected with aCSF.

To assess the involvement of the AT1 receptor in the interaction, in a second set of experiments, groups of animals received a combination of the peptides with the AT1 receptor antagonist DuP 753 in a threshold dose based on previous studies [31].

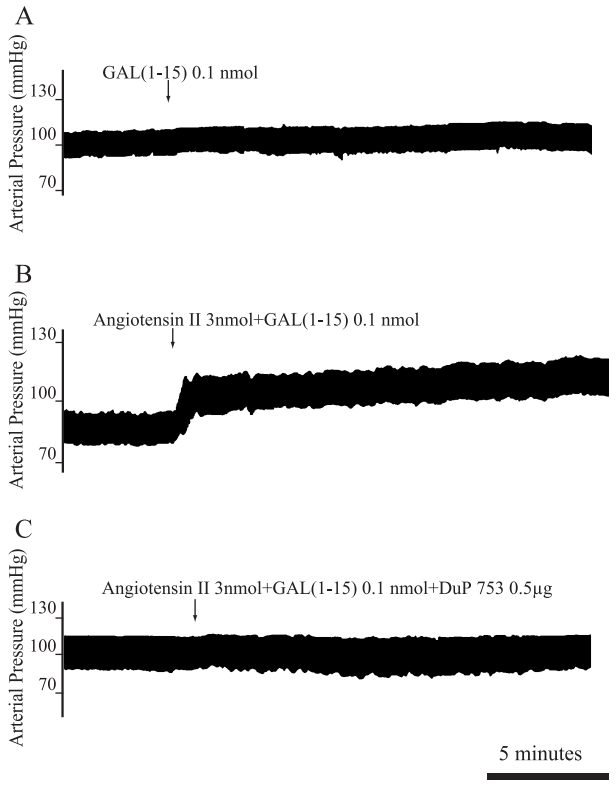


Fig. 3. Representative tracings of the effect of GAL(1–15) (A), Ang II (3 nmol/rat)+GAL(1–15) (0.1 nmol/rat) (B), and Ang II (3 nmol/rat)+GAL(1–15) (0.1 nmol/rat)+DuP 753 (0.5 µg) (C). The increase of MAP elicited by Ang II+GAL(1–15) is counteracted by DuP 753.

The threshold values for Ang II, GAL(1–29), and GAL(1–15) were determined from previous papers from our laboratory [10,32].

The total volume in all the injections was always 10 µl.

In all experiments, MAP and HR were recorded during 15 min immediately before intracisternal injections and the means of these values were used as basal values. After intracisternal injections, changes in MAP and HR were recorded during 60 min.

### 2.5. Materials

Porcine GAL(1–29) and Ang II were purchased form Peninsula Laboratories (Belmont, CA, USA). DuP 753 was kindly provided by Dupont Merck Pharmaceutical (USA). GAL(1–15) was a generous gift from Dr. N. Yanaihara (Yanaihara Institute, Shizouka, Japan).

### 2.6. Statistical analysis

The peak (maximal responses during the first 15 min) was calculated for each parameter and for each animal using an IMB-XT computer and a program developed by Guna Consult (Stockholm, Sweden). The peak effects were expressed as percent of changes from the respective mean basal values. In addition, the area created under the curve, which mainly reflects the duration of the effect under the experimental 60-min period, was calculated for each animal. The area values are expressed in arbitrary units. A non-parametric one-way analysis of variance for multiple comparisons Kruskall–Wallis followed by Dunn's posttest [33] was used for comparisons between experimental groups. The significance levels from the posttest are indicated in each case.

## 3. Results

The basal values of the MAP and HR for all groups studied were within normal limits and not significantly different form each other.

### 3.1. Interactions between Ang II and GAL(1–15) after intracisternal coadministration

The intracisternal injection of threshold doses of Ang II (3 nmol) and GAL(1–15) (0.1 nmol) alone induced a slight vasodepressor response not statistically different from the control group (Fig. 1). However, after the coinjections of Ang II with GAL(1–15), a significant vasopressor response (nonparametric ANOVA Kruskall–Wallis, $p<0.001$, followed by Dunn's posttest, $p<0.01$) was observed and it was maintained during the 60-min recording period (Fig. 1). This effect appeared 10 min after injection and reached a maximum of 33% of increase 40 min after the injection.

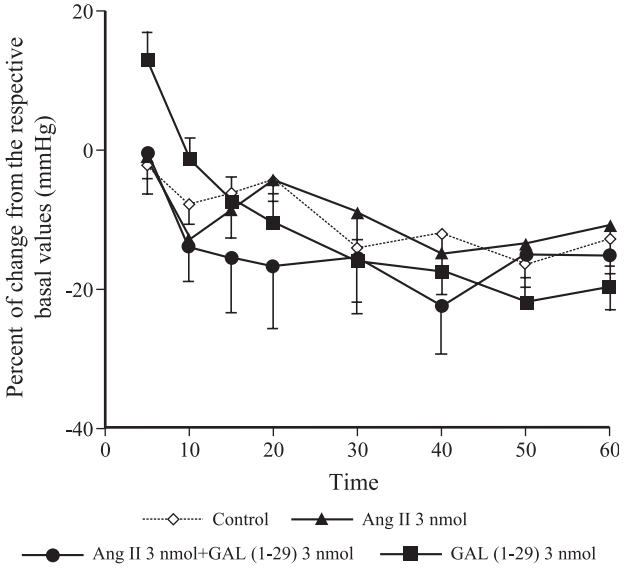


Fig. 4. Effects of intracisternal injections of Ang II (3 nmol/rat), GAL(1–29) (3 nmol/rat), GAL(1–29)+Ang II, and CSF on mean arterial blood pressure over a 60-min recording period. Mean±S.E.M. are shown as percentages of changes from the respective basal values. $n$=6–8 rats per group. The basal MAP values were: Ang II group, 84±9 mm Hg; GAL(1–29), 90±3 mm Hg; GAL(1–29)+Ang II, 70±5 mm Hg; CSF group, 74±2 mm Hg. Significances are given in Fig. 5.

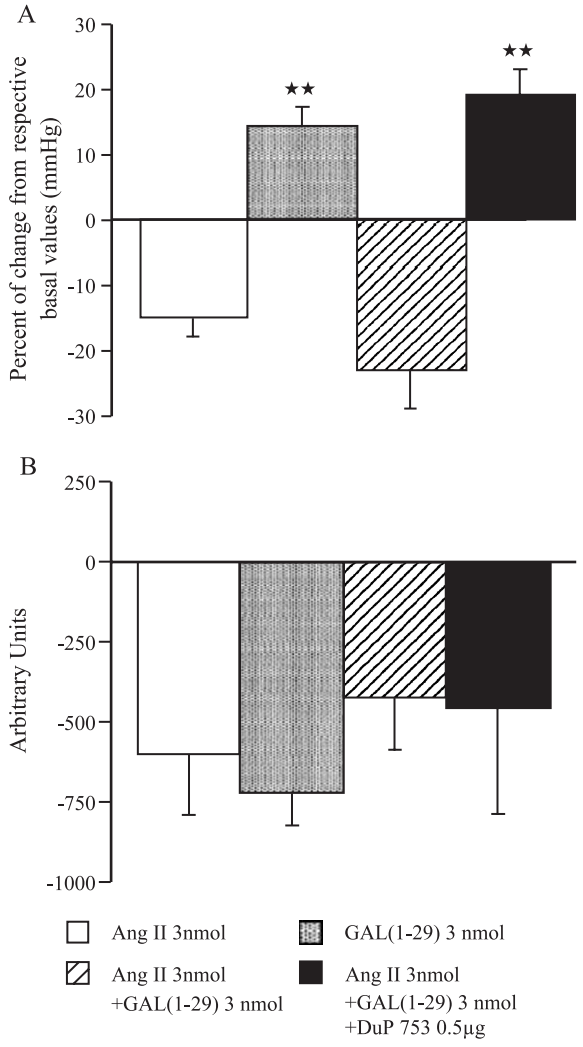


Fig. 5. Effects of intracisternal injections of Ang II (3 nmol/rat), GAL(1–29) (3 nmol/rat), GAL (1–29) (3 nmol/rat)+Ang II (3 nmol/rat), GAL(1–29) (3 nmol/rat)+Ang II (3 nmol/rat)+DuP 753 (0.5 μg), and CSF on mean arterial blood pressure. The peak effect (A) is shown as percent changes from respective basal values and the overall effects (area values) are shown (B) as absolute values in arbitrary units. Mean±S.E.M. are given. $n$=6–8 rats per group. The basal values were described in the legend of Fig. 2. GAL(1–29) (0.1 nmol/rat)+Ang II (3 nmol/rat)+DuP 753 (0.5 μg), 90±2 mm Hg. **$p$<0.01 vs. GAL (1–29) and GAL (1–29)+Ang II groups according to Kruskall–Wallis followed by Dunn's posttest.

The threshold doses of Ang II and GAL(1–15) alone produced an increase in HR as seen from the area values (Table 1). The coinjections of Ang II with GAL(1–15) induced an increase of HR during the whole period of recording as evaluated from the area values that were not significantly different from the increase of HR produced by each peptide (Table 1).

### 3.2. Interactions between Ang II and GAL(1–15) with AT1 antagonist DuP 753 after intracisternal coadministration

The AT1 antagonist DuP 753 at the threshold dose of 0.5 μg did not produce any effect at MAP or HR compared to the control group (MAP basal value DuP 753 group 85±5 mm Hg, peak value −3.4±1; HR basal value DuP 753 group 401±5 beats/min, peak value −3.1±1).

DuP 753 blocked the increase in the MAP produced by the coinjections of Ang II and GAL(1–15) (Fig. 2). This blockade was maintained for the whole recording period as observed from the area values (Fig. 2).

A representative recording illustrating the effect of DuP 753 on Ang II–GAL(1–15)-induced changes in MAP is shown in Fig. 3.

The presence of DuP 753 also counteracted the tachycardic response produced by Ang II and GAL(1–15). The HR changes observed after the coinjection of Ang II–GAL(1–15)–DuP 753 were not significantly different from the control values (Table 1).

### 3.3. Interactions between Ang II and GAL(1–29) after intracisternal coadministration

Intracisternal injection of GAL(1–29) elicited a transient increase of MAP followed by a rapid decrease. This early increase of MAP appeared 5 min after the injections and disappeared very rapidly, and from the 10th minute, MAP decreased progressively, although it was not statistically different from the control group (Fig. 4).

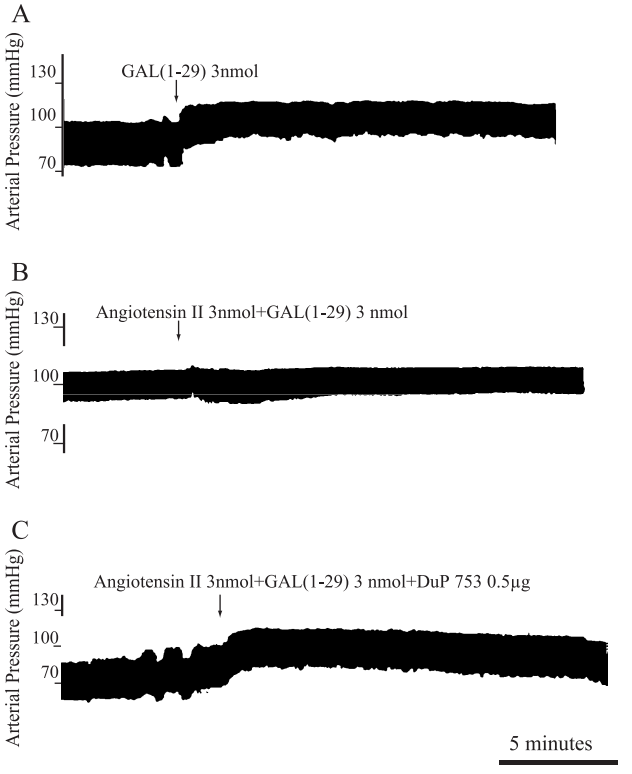


Fig. 6. Representative tracings of the effect of GAL(1–29) (3 nmol/rat) (A) and in rats given Ang II (3 nmol/rat)+GAL(1–29) (3 nmol/rat) (B), and Ang II (3 nmol/rat)+GAL(1–29) (3 nmol/rat)+DuP 753 (0.5 μg) (C). The increase of MAP elicited by GAL(1–29) disappears in the presence of Ang II, but is observed again with DuP 753.

The intracisternal coinjections of Ang II with GAL(1–29) did not produce any significant effects in MAP as compared with the control values and Ang II itself (Fig. 4). However, the transient vasopressor response produced by GAL(1–29) alone disappeared in the presence of Ang II (Fig. 5).

GAL(1–29) injections produced a slight but significant tachycardic effect that was not modified in the presence of Ang II (Table 1).

### *3.4. Interactions between Ang II and GAL(1–29) with AT1 antagonist DuP 753 after intracisternal coadministration*

The coinjections of Ang II with GAL(1–29) and with DuP 753 produced an increase in the MAP that was observed during the first 15 min (Fig. 5). This transient vasopressor response was not different from the MAP increase produced by GAL(1–29) alone (Fig. 5).

A representative recording illustrating the effect of DuP 753 on Ang II–GAL(1–29)-induced changes in MAP is shown in Fig. 6.

The presence of DuP 753 did not modify the tachycardic response produced by Ang II and GAL(1–29) (Table 1).

## 4. Discussion

The findings presented in this paper give indications for the existence of a differential modulatory effect of Ang II with GAL(1–15) and GAL(1–29) on central blood pressure regulation. We further provide the first demonstration that this modulation is dependent on the activity of the angiotensin AT1 receptor subtype.

With regard to Ang II/GAL(1–15) interaction, one possible explanation for the present observations may be that simultaneous activation of Ang II receptors and postulated GAL(1–15) receptors subtypes leads to an increased transmission over the galanin fragment receptor via a receptor–receptor interaction, favoring transmission over the galanin fragment receptor.

These results are in agreement with previous reports showing that threshold (with respect to their effects on mean arterial blood pressure) doses of intracisternally administered Ang II or GAL(1–15) induced a weak vasodepressor response not significantly different from the control group. The discovered vasopressor response to coadministration of Ang II and GAL(1–15) had a magnitude similar to the maximal pressor effect of GAL(1–15) found when given alone [10].

The Ang II receptor involved in this modulation may be of the AT1 receptor subtype since the AT1 antagonist DuP 753 fully blocked this effect. In fact, the AT1 receptor subtype is the predominant Ang II receptor in all the areas involved in cardiovascular regulation, including the NTS and the rostral and caudal parts of the VLM in the brainstem [16]. Moreover, studies showing increased density or activity of AT1 receptors in cardiovascular regions in various genetic and experimental hypertension models have implicated central AT1 receptors as causally involved in the development and maintenance of hypertension [29] when the postulated interaction between AT1/galanin fragment receptors may contribute.

Until now, three cloned receptor subtypes for galanin–GAL-R1, GAL-R2, and GAL-R3—have been described [4,6]. In situ hybridization studies have reported that GAL-R1 and GAL-R2 are expressed in nuclei involved in cardiovascular regulation. These two receptors are known to show a higher affinity for GAL(1–29) than for galanin-N terminal fragments such as GAL(1–15) [2]. However, the presence of specific binding sites for this galanin fragment in the nuclei involved in central cardiovascular regulation has been described [34]. In the brainstem, a high density of these GAL(1–15) binding sites appears within the NTS, but not in the VLM [34], and presumably this may represent the primary site for the GAL(1–15) action demonstrated in the present study. In view of all these data, it may be suggested that the discovered interaction between GAL(1–15) and AT1 receptors subtypes take place in the NTS. It must be emphasized that there exists a codistribution of GAL(1–15) and AT1 receptors binding sites in this area [18,34], and thus it seems possible that AT1/GAL(1–15) receptor interactions can activate the sympathetic system by their coexistence in NTS neurons. However, we cannot exclude that other nuclei might be involved since the intracisternal injections influence all the cardiovascular brainstem regions rather than specific nuclei. In fact, the intracisternal injection of GAL(1–15) induces c-Fos expression in the VLM and other medullary regions such as the ambiguus nucleus [35] where Ang II receptors have been described [18].

Nevertheless, network interactions in the control of sympathetic outflow cannot be excluded as an alternative explanation of the present findings with subthreshold doses of GAL(1–15) and Ang II. The vasopressor response observed after the coinjection of GAL(1–15) and Ang II could be a simultaneous activation of the sympathetic nervous pathways by both peptides. In fact, it should be considered that Ang II is well known to increase MAP due to an activation of AT receptors in the brainstem [16]. Furthermore, recent studies have shown that vasopressor actions elicited by central administration of GAL(1–15) are blocked by the peripheral pretreatment with the β-receptor antagonist, propranolol, but not the peripheral pretreatment with the cholinergic antagonist, atropine, suggesting, at least at heart level, an activation of sympathetic nerve activity rather than inhibition of parasympathetic outflow [12]. Thus, the sympathetic rather than the parasympathetic outflow seems to be involved in the present findings.

Another mechanism such as the involvement of other peptides or humoral factors cannot be excluded from the pressor response observed, but probably their involvement depends on the Ang II/GAL(1–15) interaction observed.

As described in previous reports, the intracisternal injection of GAL(1–29) elicited a transient clear-cut

increase of MAP followed by a rapid decrease, although not significantly different from the control group [10]. However, the transient vasopressor response produced by intracisternal GAL(1–29) alone disappeared in the presence of Ang II also given intracisternally. Moreover, this transient modulation by Ang II of GAL(1–29)-mediated vasopressor response was blocked by the AT1 antagonist DuP 753.

Several authors have suggested that intracisternal GAL(1–29) actions might be mediated through the parasympathetic pathway [35,36]. Thus, the changes in MAP and the tachycardia elicited by intracisternal GAL(1–29) are modified by the pretreatment with atropine but not with propranolol showing the involvement of parasympathetic pathways in the central effect of this peptide [12]. However, intracisternal injections of GAL(1–29) also induced c-Fos expression in areas responsible for the sympathetic drive such as A1 and C1 areas where a high density of GAL(1–29) binding sites exist [35], suggesting the involvement of these regions in the actions modulated by GAL(1–29) [35]. Thus, it is possible that the interaction between GAL(1–29) and Ang II could reflect an interaction in the central circuits controlling the sympathetic outflow. This will explain the fact that the presence of Ang II blocked the vasopressor response produced by intracisternal GAL(1–29) 5 min after the injection.

When analyzing the HR response in the area values, a long-lasting tachycardic response after the intracisternal administration of Ang II and GAL(1–15) alone was observed in agreement with previous reports. Furthermore, the coadministration of Ang II with GAL(1–15) also resulted in a tachycardic response that was maintained in the whole period of record. Endogenous Ang II exerts a tonic inhibitory modulation on the baroreceptor reflex [26] and GAL(1–15) decreases baroreceptor reflex sensitivity [13]; therefore, it may be suggested that the increase in HR observed is a consequence of an inhibition of the baroreceptor reflex. This is true also after the combined treatment leading to vasopressor responses, indicating that the postulated receptor–receptor interaction in the NTS now leads to an enhanced inhibition of the baroreceptor reflex. Our results confirm and extend the involvement of AT1 receptors also in HR regulation since the presence of DuP 753 also counteracted the tachycardic response produced by Ang II and GAL(1–15). In fact, the involvement of AT1 receptors, but not AT2 receptors, has been demonstrated in the inhibition of the baroreceptor reflex by Ang II [27]. Moreover, Ang II induces the expression of the c-*fos* gene in the NTS via activation of the AT1 receptors [28].

As to HR responses with GAL(1–29) injections, the tachycardic action was not modified by the presence of Ang II or the AT1 antagonist. Since GAL(1–29) did not block the bradycardic response elicited by intravenous L-phenylephrine [13], it may be suggested that the effect at HR by GAL(1–29) does not change the activity of the baroreceptor reflex. This can also explain the absence of interaction between GAL(1–29) and Ang II in central HR regulation.

In conclusion, the cardiovascular analysis shows vasopressor responses upon intracisternal coinjections of subthreshold doses of GAL(1–15) and Ang II that may be explained by postulated synergistic signalling interactions between AT1 and GAL(1–15) receptors, such synergistic interaction could not be found for GAL(1-29)/Ang II.

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