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The galanin receptor antagonist M40 blocks the central cardiovascular actions of the galanin N-terminal fragment (1–15)

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Abstract

It has been shown that galanin plays a role in central cardiovascular regulation. Galanin administered centrally induces an increase of heart rate and a weak vasodepressor response, whereas the N-terminal galanin fragment (1–15) elicits vasopressor effects and tachycardia. Furthermore, it has been shown that galanin-(1–15), but not galanin-(1–29), decreases the baroreceptor reflex sensitivity. Since these data demonstrate that both galanin and its N-terminal fragment (1–15) exert a different modulation on central cardiovascular control, the aim of this work has been to study if the specific galanin receptor antagonist Galanin-(1–12)-Pro-(Ala-Leu)$_2$-Ala]-amide (M40) could modulate their cardiovascular actions. Urethane anaesthetized rats were injected intracisternally and the changes in mean arterial pressure and heart rate were monitored. Two doses of M40 alone have been tested for their cardiovascular effects. With the dose of 1.0 nmol, a significant tachycardia was observed ($P < 0.001$), but 0.1 nmol was ineffective. This suggests a possible agonistic effect for the higher doses of M40. The galanin receptor antagonist M40 at the dose of 0.1 nmol failed to modify the weak vasodepressor effects and tachycardia induced by 3.0 nmol of galanin-(1–29). However, the same dose completely blocked the vasopressor and tachycardic responses elicited by 3.0 nmol of galanin-(1–15). These data show that M40 differentially counteracts the central cardiovascular responses of the galanin fragment and give a functional support for the existence of galanin receptor subtypes within the brainstem. Therefore, the present findings can be explained on the basis that the cardiovascular actions of galanin-(1–29) could be mediated by one type of galanin receptor, whereas a galanin receptor subtype that recognizes N-terminal fragments of galanin may mediate the actions of galanin-(1–15).

Keywords: Galanin; Galanin-(1–15); Galanin receptor; Galanin receptor antagonist; Cardiovascular; Neuropeptide; Brain

1. Introduction

Galanin is a 29-aminoacid peptide originally isolated from the porcine intestine (Tatemoto et al., 1983) and widely distributed in the brain (Skofitsch and Jacobowitz, 1985; Melander et al., 1986). The distribution of specific binding sites for [^{125}I]galanin is well characterized. It is similar to the distribution of galanin immunoreactive neurons (Skofitsch et al., 1986; Melander et al., 1988) and to the distribution of the galanin receptor mRNA levels, which recently have been mapped out (Gustafson et al., 1996). Galanin has been involved in multiple physiological functions including neuroendocrine control (Melander et al., 1987; Merchentaler, 1991), food intake (Crawley et al., 1990), nociception (Verge et al., 1993), memory and learning (Ogren et al., 1992), and central cardiovascular control (Härfstrand et al., 1987; Hedlund et al., 1991).

Structure–activity studies have shown that in the brain N-terminal fragments of galanin are biologically active, whereas C-terminal fragments are inactive (Fisone et al., 1989a; Lagny-Pourmir et al., 1989; Land et al., 1991). In binding studies, the N-terminal fragments displace [^{125}I]galanin (Fisone et al., 1989a,b; Yanaihara et al., 1991; Girotti et al., 1993) and they act as agonists in several physiological functions (Bartfai et al., 1993a; Crawley, 1995), suggesting a receptor mediated action. Recently, the existence of specific binding sites for the N-terminal galanin fragment 1–15 has been described within the central nervous system, also in areas lacking [^{125}I]galanin

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binding sites, giving indications for the existence of a galanin receptor subtype only recognizing N-terminal galanin fragments (Hedlund et al., 1992). These binding sites have inter alia been shown to be present in the hypothalamus and the brainstem, where a cluster of the N-terminal galanin-(1–15) binding sites is located in the dorsal part of the nucleus tractus solitarius, where [^{125}I]galanin-(1–29) binding sites also are present (Hedlund et al., 1992).

Since intracisternal injections of galanin-(1–15) induce vasopressor responses with tachycardia (Narváez et al., 1994), an opposite effect to that induced by galanin-(1–29) (Härfstrand et al., 1987; Hedlund et al., 1991), it may be proposed that the central cardiovascular effects of galanin and its N-terminal fragment could be mediated by different receptor subtypes. Furthermore, galanin-(1–15) was shown to decrease baroreceptor sensitivity, whereas galanin-(1–29) had no effect in this regard (Díaz et al., 1996), and both peptides modulate differentially the autonomic nervous system (Narváez et al., 1998). Also, both galanin molecules interact with 5-HT$_{1A}$ receptors in a distinct way (Hedlund et al., 1991, 1994; Narváez et al., 1994). Taken all these evidences together, it may be suggested that both molecules might act through different receptor subtypes recognizing galanin and/or its N-terminal fragments.

Many attempts have been made to demonstrate the existence of galanin receptor subtypes by the use of specific receptor antagonists. These antagonists include M15 [Galanin-(1–12)-Pro-Substance P-(5–11)] (Bartfai et al., 1991), M35 [Galanin-(1–12)-Pro-Bradykinin-(2–9)] (Wiesenfeld-Hallin et al., 1992), M40 [Galanin-(1–12)-Pro-(Ala-Leu)$_2$-Ala]-amide] (Bartfai et al., 1993b), C7 [Galanin-(1–12)-Pro-Spantide] (Crawley et al., 1993), M32 [Galanin-(1–12)-Pro-Neuropeptide Y-(25–36)] (Corwin et al., 1993), or galparan [Galanin-(1–12)-Pro-Mastoparan] (Langel et al., 1996). By the use of these molecules several putative galanin receptor subtypes have been described (Kask et al., 1995). However, in view of the chimerical nature of these peptide antagonists they could theoretically interact with receptors apart from galanin receptors and thus the interpretation of the data is difficult.

This is not the case for the galanin receptor antagonist M40, since there is no receptors for its C-terminal fragment. It seems as if this fragment interacts with the galanin receptor surface or surrounding area of the membrane to increase the affinity of the antagonist and thus to increase the ability to displace [^{125}I]galanin (Bartfai et al., 1993b). Because of these characteristics, M40 has been used as the galanin receptor antagonist in the present experiments.

Thus, the aim of this work is to study if the galanin receptor antagonist M40 could differentially modulate the central cardiovascular responses of galanin-(1–29) and of its N-terminal fragment (1–15). In fact, the results obtained give functional evidence for the existence of galanin receptor subtypes involved in central cardiovascular control.

2. Materials and methods

2.1. Animals

Male specific pathogen-free Sprague–Dawley rats (200–250 g b.w., CRIFFA, Barcelona, Spain) were used. The animals were kept in the animal quarters under regular lighting conditions (lights on at 06:00 h and off at 20:00 h) and had free access to food pellets and tap water.

2.2. Cardiovascular experiments

The animals were anaesthetized with urethane (1.1 g/kg b.w.) and immediately tracheotomized. The femoral artery was cannulated with a plastic catheter containing heparin (50 IU/ml 0.9% NaCl w/v) to record blood pressure and heart rate, and the animal was placed in a stereotaxic frame. The head 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, the body temperature was maintained at 37.5 ± 0.5°C by means of a thermostatic blanket.

Mean arterial blood pressure and heart rate were recorded by connecting the femoral catheter to a Statham-type transducer linked to a computerised data acquisition system (MacLab, AD Instruments). When the animals were stabilized, the cardiovascular parameters were recorded during 15 min, immediately before intracisternal injections, and these data were used as basal values. The modifications of mean arterial pressure and heart rate were recorded during 60 min after injections.

2.3. Intracisternal injections

Intracisternal injections were made 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 5.8 mM D-glucose (pH 7.4)]. The volume injected was 10 μl in a period of 10–12 s.

To study if M40 alone exerts any effect on central cardiovascular parameters, groups of rats ($n = 5$–7) received intracisternal injections of M40 at doses of 0.1 or 1.0 nmol. These doses of M40 have been shown to block the feeding effects of galanin in the hypothalamus, having no effect by themselves (Corwin et al., 1993; Crawley et al., 1993).

To investigate the possible modulation of M40 on the central cardiovascular effects of galanin-(1–15), different groups of rats ($n = 7$–8) were injected intracisternally with a maximal dose of galanin-(1–15) (3.0 nmol; Narváez et

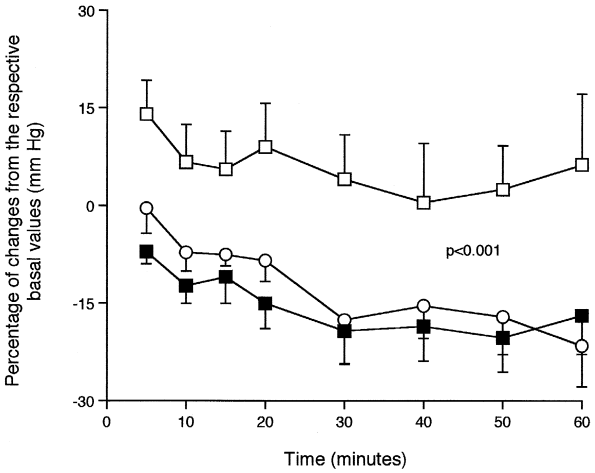

Fig. 1. Effects of intracisternal injections of 3.0 nmol galanin-(1–15) (open squares) or 3.0 nmol galanin-(1–15) + 0.1 nmol M40 (black squares) on mean arterial pressure over the 60-min recording period. Open circles represent the control aCSF group. The percentage of changes from the respective basal values are shown as means ± S.E.M. ($n = 7–8$ rats per group). Basal values were: control group, 89 ± 5 mm Hg; galanin-(1–15), 85 ± 3 mm Hg; galanin-(1–15) + M40, 90 ± 10 mm Hg. $P < 0.001$ is the significance level of galanin-(1–15) alone vs. control group and vs. galanin-(1–15) + M40 group during the whole period of recording (Dunn's test).

al., 1994), or coinjected with this dose of galanin-(1–15) and a subthreshold dose of M40 (0.1 nmol).

Since galanin-(1–29) induces a tachycardia independent of dose (Härfstrand et al., 1987; Narváez et al., 1994), an equimolar dose of galanin-(1–29) (3.0 nmol) has been used in these experiments. Thus, to study the possible modulation of the cardiovascular effects of galanin-(1–29) by the antagonist M40, groups of animals ($n = 7–8$) were injected with 3.0 nmol of galanin-(1–29) alone, or coinjected with the same dose of galanin-(1–29) and a subthreshold dose of M40 (0.1 nmol).

A group of animals ($n = 6$) received intracisternal injections of aCSF alone and were used as a control group.

2.4. Materials

Porcine galanin-(1–29) and the galanin receptor antagonist M40 were purchased from Peninsula Laboratories, Belmont, CA, USA. Galanin-(1–15) was a generous gift from Dr. N. Yanaihara (Yanaihara Institute, Shizouka, Japan).

2.5. Statistical analysis

The cardiovascular changes elicited by both doses of M40 and by the control aCSF group were analyzed by means of a non-parametric one-way analysis of variance for multiple comparisons (Dunn's test) (Hollander and Wolfe, 1973). The same statistical protocol was used also to evaluate the results in the other cardiovascular experiments. The significance levels are indicated in each case.

3. Results

3.1. Intracisternal injections of galanin-(1–15) and M40

Intracisternal injections of 3.0 nmol of the N-terminal galanin fragment (1–15) induces a significant vasopressor effect ($P < 0.001$ vs. control group) that appears early after the injections and is maintained with a similar potency during the whole period of recording (Fig. 1). However, after the coinjections of galanin-(1–15) with the antagonist M40 the vasopressor response completely disappears (Fig. 1). This blocking effect is highly significant ($P < 0.001$ vs. galanin-(1–15) alone) and maintains blood pressure at a similar level as observed in the control group. Together with the vasopressor action, galanin-(1–15) increases heart rate significantly ($P < 0.001$ vs. control group), this effect reaches a plateau 20 min after injections, which is observed during the 60 min recording period (Fig. 2). The coinjection of the antagonist M40 blocks also the increase of the heart rate and the response observed is not different from the control group (Fig. 2). This blocking effect is observed early on and is maintained during the whole period of recording.

In these experiments, the galanin receptor antagonist M40 injected alone at doses of 1.0 nmol produces a significant increase on mean arterial pressure at 50 min after intracisternal injection (Table 1). Also a significant increase of heart rate ($P < 0.001$) appears in the first 15 min after the injections (Fig. 3); this is maintained during the whole period of recording. The tachycardia induced by

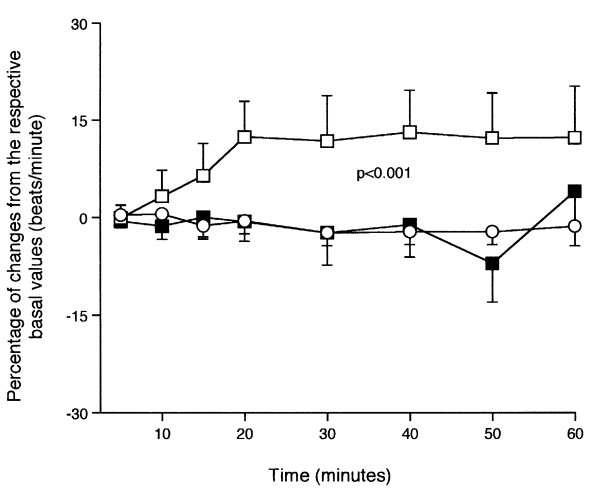

Fig. 2. Effects of intracisternal injections of 3.0 nmol galanin-(1–15) (open squares) or 3.0 nmol galanin-(1–15) + 0.1 nmol M40 (black squares) on heart rate over the 60-min recording period. Open circles represent the control aCSF group. The percentage of changes from the respective basal values are shown as means ± S.E.M. ($n = 7–8$ rats per group). Basal values were: control group, 383 ± 20 beats/min; galanin-(1–15), 380 ± 13 beats/min; galanin-(1–15) + M40, 386 ± 22 beats/min. $P < 0.001$ is the significance level of galanin-(1–15) alone vs. control group and vs. galanin-(1–15) + M40 group during the whole period of recording (Dunn's test).

Table 1
Mean arterial pressure changes after intracisternal injections of the galanin receptor antagonist M40 at different doses

Time (min)	M40 1.0 nmol	M40 0.1 nmol
5	−4.7±3	−8.4±1
10	−7.6±4	−8.3±2
15	−10.3±4	−10.5±4
20	4.6±10[a]	−10.5±3
30	−5.8±6	−10.2±4
40	2.0±6[a]	−7.2±5
50	15.0±10[b]	−2.2±7
60	11.0±9[b]	0.2±3

Data are expressed as means±S.E.M. of the percentage of changes from the respective basal values. Basal values were M40 1.0 nmol, 89±10 mm Hg; M40 0.1 nmol, 86±8 mm Hg; control group, 95±5 mm Hg.
[a] $P < 0.05$ vs. control group;
[b] $P < 0.01$ vs. control group ($n = 5–7$).

the highest dose of M40 alone (7.9 ± 5%, mean ± S.E.M) is statistically similar to that observed at 15 min after galanin-(1–29) or galanin-(1–15) alone (4.2 ± 2% and 6.5 ± 4.0%, respectively, mean ± S.E.M.) This effect is maintained and at the end of the recording period is even significantly higher than those observed with the two galanin peptides ($P < 0.05$). No significant changes in mean arterial pressure (Table 1) and heart rate (Fig. 3) are observed with the dose of 0.1 nmol.

3.2. Intracisternal injections of galanin-(1–29) and M40

Intracisternal injections of 3.0 nmol of galanin-(1–29) elicit a transient increase of mean arterial pressure followed by a rapid decrease (Fig. 4). This early increase of mean arterial pressure appears 5 min after the injections and disappears very rapidly, and from the tenth minute a progressive decrease in mean arterial pressure is observed reaching a plateau at the 30 min time interval (Fig. 4). The coinjection of 0.1 nmol of M40 does not modify the changes of mean arterial pressure induced by galanin-(1–29); only a slight decrease in the early vasopressor response to galanin-(1–29) is observed (Fig. 4).

Galanin-(1–29) elicits an increase of heart rate. This tachycardia appears 5 min after the injections ($P < 0.05$) but after 15 min it is not statistically different from controls (Fig. 5). When galanin-(1–29) is coinjected with 0.1

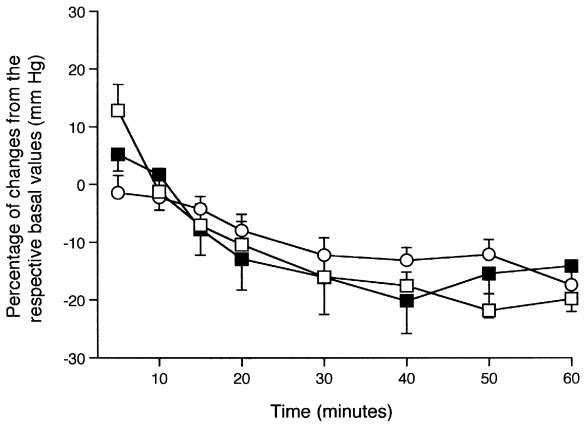

Fig. 4. Effects of intracisternal injections of 3.0 nmol galanin-(1–29) (open squares) or 3.0 nmol galanin-(1–29)+0.1 nmol M40 (black squares) on mean arterial pressure over the 60-min recording period. Open circles represent the control group. The percentage of changes from the respective basal values are shown as means±S.E.M. ($n = 7–8$ rats per group). Basal values were: control group, 86±6 mm Hg; galanin-(1–29), 90±3 mm Hg; galanin-(1–29)+M40, 88±10 mm Hg.

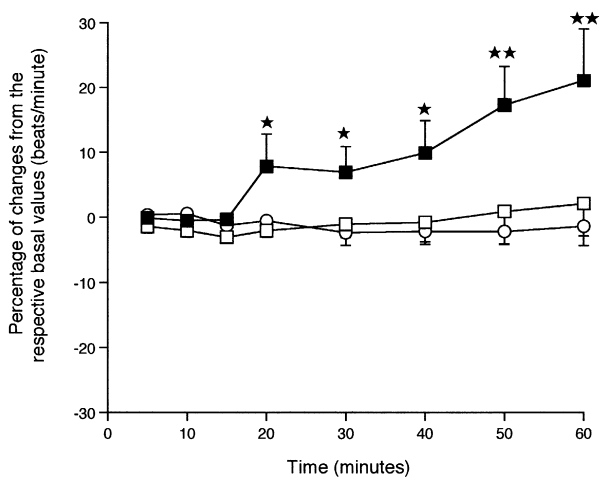

Fig. 3. Effects of intracisternal injections of M40 alone on heart rate. Doses of 0.1 nmol (open squares) and 1.0 nmol (dark squares) have been studied. Control group received aCSF alone (open circles). The percentage of changes from the respective basal values are shown as means± S.E.M. ($n = 5–7$ rats per group). Basal values were: control group, 383±19 beats/min; M40 1.0 nmol, 371±23 beats/min; M40 0.1 nmol, 386±10 beats/min. ★★$P < 0.01$ and ★★★$P < 0.001$ vs. M40 0.1 nmol and vs. control group (Dunn's test).

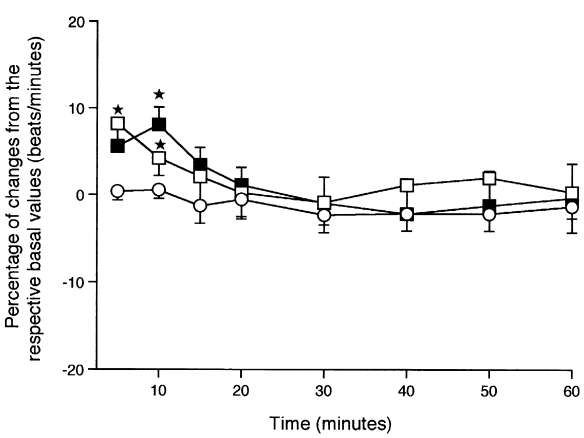

Fig. 5. Effects of intracisternal injections of 3.0 nmol galanin-(1–29) (open squares) or 3.0 nmol galanin-(1–29)+0.1 nmol M40 (black squares) on heart rate over the 60-min recording period. Open circles represent the control group. The percentage of changes from the respective basal values are shown as means±S.E.M. ($n = 7–8$ rats per group). Basal values were: control group, 420±13 beats/min; galanin-(1–29), 420±13 beats/min; galanin-(1–29)+M40, 402±22 beats/min. ★$P < 0.05$ vs. control group (Dunn's test).

nmol of the galanin receptor antagonist M40 the increase in heart rate is still significantly different from the control group value ($P < 0.05$). In this case, the heart rate response is of a similar potency to that observed after galanin-(1–29) alone (Fig. 5). Thus, M40 fails to block the central cardiovascular effects induced by galanin-(1–29).

4. Discussion

Different galanin receptor antagonists have been used for the identification of putative galanin receptor subtypes. These molecules are mostly chimeras containing C-terminal fragments from other bioactive peptides (Bartfai et al., 1993a) that usually have a role in central cardiovascular regulation, such as substance P, Neuropeptide Y or Bradykinin. Thus, these chimerical antagonists could theoretically stimulate different receptors than galanin and it may be difficult to explain how they block the galanin receptor. However, the antagonist M40 carries a C-terminal sequence which is not thought to be a ligand at any known receptor (Bartfai et al., 1993b) and therefore the data obtained can be confined to the effects of this antagonist on the galanin receptors. Furthermore, M40 is shown to act as a high affinity ligand for galanin receptors in the hippocampus, hypothalamus and spinal cord, although its affinity is somewhat lower than that of the endogenous ligand galanin (Bartfai et al., 1993b).

Initially, based on the pharmacological effects of M40, two galanin receptor subtypes were described. One receptor was located in the hypothalamus and in the hippocampus, where M40 act as a potent antagonist, and a second subclass is located in the pancreas where M40 acts as a weak agonist (Bartfai et al., 1993b). However, although M40 displaces [^{125}I]galanin from its binding sites in brain tissue, at the spinal cord level even a 1000-fold excess of M40 does not fully displace [^{125}I]galanin. Furthermore, M40 does not antagonize the inhibitory effects of galanin on the forskolin-stimulated accumulation of 3′,5′-cAMP in the Rin m5F insulinoma cells (Bartfai et al., 1993b). The failure of antagonistic effects of M40 and even the presence of agonistic effects have also been described in other systems (Billecocq et al., 1994; Xu et al., 1995; Papas and Bourque, 1997; Kinney et al., 1998). All these data suggest the existence also of an intermediate subclass of galanin receptor in the central nervous system, different from the pancreatic or the hypothalamic subclasses, that may be located mainly in the spinal cord. Also different types of galanin receptors have been described within the hypothalamus and in the basal forebrain using the galanin receptor antagonists M15 or M35 (Wynick et al., 1993; Deecher et al., 1995).

In this paper, we demonstrate that the galanin receptor antagonist M40 blocks the central cardiovascular responses induced by the N-terminal galanin fragment (1–15), but exerts no effect on the cardiovascular responses induced by galanin-(1–29). Although in several systems long N-terminal fragments of galanin act as full or partial galanin agonists (Bartfai et al., 1993a; Crawley, 1995), it is shown in these experiments that while galanin-(1–29) has no effect on mean arterial pressure, galanin-(1–15) elicits a significant vasopressor action. These different effects on the central cardiovascular regulation have been reported previously and also it has been shown that galanin-(1–15) counteracts the cardiovascular responses of galanin-(1–29) (Narváez et al., 1994). Furthermore, galanin-(1–15) decreases the sensitivity of the baroreceptor reflex, whereas galanin-(1–29) exerts no effects (Díaz et al., 1996). It seems that both peptides modulate differentially the autonomic nervous activity (Narváez et al., 1998). In this way, differential effects of galanin-(1–29) and galanin-(1–15) have been reported on animal behavior (Przewlocka et al., 1995). In addition, both molecules modulates differentially the central cardiovascular responses mediated by 5-HT$_{1A}$ receptors (Hedlund et al., 1991; Narváez et al., 1994), the 5-HT$_{1A}$ binding in the dorsal hippocampus (Hedlund et al., 1994), and the inhibition of glutamate release in the arcuate nucleus (Kinney et al., 1998).

From all these data, it could be suggested at least from a functional point of view that a specific galanin receptor subtype might mediate the central cardiovascular actions of the N-terminal galanin-(1–15) fragment where M40 acts as a full antagonist.

However, M40 does not antagonize the cardiovascular effects elicited by galanin-(1–29). Galanin-(1–29) induces a significant increase of heart rate with a weak decrease of mean arterial pressure (Härfstrand et al., 1987; Hedlund et al., 1991; Narváez et al., 1994). It is noticeable that in the present experiments, galanin-(1–29) elicits a short and transient increase of mean arterial pressure followed by a rapid decrease. This effect has not been described earlier and it can be explained because in the present animals, blood pressure has been recorded from the femoral artery instead of the carotid artery, where modifications of baroreceptor reflexes could be initiated by surgery.

The tachycardia elicited after galanin-(1–29) and M40 coinjections is of the same potency and magnitude as that induced by galanin-(1–29) alone, and the changes of the mean arterial pressure are not statistically different from the control group. Only a weak antagonistic effect on the early transient vasopressor response is observed. It is unlikely that administration of both substances at the same time distort the possible antagonism of M40 on the cardiovascular effects of galanin-(1–29) since it has been demonstrated that the M40 possesses the same effect when injected before or after galanin (Corwin et al., 1993). Thus, it can be suggested that from a functional point of view M40 does not recognize the galanin receptor subtype that mediates the central cardiovascular actions of galanin-(1–29).

In these experiments, intracisternal injections of M40 alone have no effects on mean arterial pressure, but with

the higher dose (1.0 nmol) an increase of the heart rate is observed. Although high doses of M40 into the paraventricular hypothalamic nucleus cause a selective dose-dependent reduction of fat intake (Leibowitz and Kim, 1992), the doses selected in these experiments have no effect on feeding behavior by themselves (Corwin et al., 1993, 1995; Crawley et al., 1993). Furthermore, in other reports, the blocking effect of M40 on galanin biological actions is observed at higher doses than these used in this paper (McDonald and Crawley, 1996; Consolo et al., 1998; McDonald et al., 1998). Therefore, it seems as if M40 at the dose of 1.0 nmol mimics the cardiovascular effects of galanin-(1–29). Partial agonist effects of M40 on galanin receptors have also been reported by other authors (see above and Billecocq et al., 1994; Xu et al., 1995; Papas and Bourque, 1997; Kinney et al., 1998).

Taken all these data together, it may be suggested that the cardiovascular actions of galanin-(1–29) and galanin-(1–15) could be mediated through different galanin receptor subtypes. Thus, the existence of at least two galanin receptors could be functionally supported, one where M40 acts as an agonist or a weak antagonist and which would recognize galanin-(1–29) and a second subclass where M40 is functionally an antagonist and which would exclusively recognize N-terminal galanin fragments.

In conclusion, the data presented in this paper demonstrate that M40 blocks selectively the central cardiovascular responses induced by galanin-(1–15) having no effect on the responses elicited by galanin-(1–29). This suggests that the actions exerted on central cardiovascular control by galanin and the N-terminal galanin fragments molecules might be mediated by different receptor subclasses. However, further studies are needed for the complete identification of these receptor subtypes.

Acknowledgements

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