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Effect of β-adrenoceptors on the behaviour induced by the neuropeptide glutamic acid isoleucine amide

Mariela E. Sánchez-Borzone, Andrés Attademo, Gustavo Baiardi, María Ester Celis *

Laboratorio de Ciencias Fisiológicas, Cátedra de Bacteriología y Virología Médica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Santa Rosa 1085, Córdoba C.P. 5000, Argentina

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Abstract

Excessive grooming behaviour is induced by intracerebroventricular injections of the neuropeptide glutamic acid isoleucine amide (neuropeptide-EI), via the activation of A-10 dopaminergic neurons and the noradrenergic system. Our object was to study the latter system involved in these behaviours, using male Wistar rats weighing 250–300 g with i.c.v. implants. The results show that all the adrenoceptor antagonists "per se" do not affect excessive grooming behaviour or motor activity. Intracerebroventricular administration of propranolol, a general β-adrenoceptor antagonist, before neuropeptide-EI, inhibited the induced excessive grooming behaviour in a dose dependent manner. Metoprolol, a β_1-adrenoceptor antagonist, also blocked this behaviour. However, intracerebroventricular injections of phentolamine, an α-adrenoceptor antagonist, and ((±)-1-[2,3-(Dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol), a β_2-adrenoceptor antagonist, had no effect on the behaviour induced by neuropeptide-EI induced behaviour for any of the doses tested. On the other hand, isoproterenol, a general β-adrenoceptor agonist and dobutamine, a β_1-adrenoceptor agonist, both elicited similar behaviours as those induced by neuropeptide-EI. These results support the hypothesis that a relationship exists between neuropeptide-EI and β-adrenoceptors, more specifically the β_1-adrenoceptor, as found with other similar endogenous peptides such as neurotensin, cholecystin, substance P and α-melanocyte stimulating hormone. Hence, neuropeptide-EI could probably be exerting a neuromodulating effect on the central nervous system.

Keywords: Neuropeptide EI; Excessive grooming behaviour; Noradrenergic system; i.c.v. injection

1. Introduction

The interaction between neuropeptides and neurotransmitters is considered to play an important role in the regulation of motor function and behaviour (Torre and Celis, 1986, 1988, 1989; Gonzalez et al., 1997, 1998; Sanchez et al., 1997) Both the mammalian melanin-concentrating hormone and the neuropeptide glutamic acid isoleucine amide (neuropeptide-EI) are encoded by a precursor of 165 amino acids. In rodents these peptides are predominantly expressed in the perikarya of the lateral hypothalamus and the subzona incerta, and project widely throughout the central nervous system (CNS) (Skofitsch et al., 1985; Bittencourt et al., 1992). Since discovered, this widespread distribution has suggested that these peptides are probably involved as neuromodulators/neurotransmitters in a number of neural functions (Baker, 1994; Nahon, 1994).

Regarding neuropeptide-EI, previous results indicate that it is involved in behaviour (Sanchez et al., 1997; Gonzalez et al., 1998), feeding (Maulon-Feraille et al., 2002) and reproduction (Attademo et al., 2004, 2006). Our laboratory demonstrated that intracerebroventricular administration (i.c.v.) of neuropeptide-EI could induce excessive grooming behaviour (EGB) and increase motor activity (MA), measured by the addition of rearing and crossing the cage (Sanchez et al., 2001; Berberian et al., 2002). It is well know that grooming has a non-stressing effect in rodents. Spontaneous grooming behaviour can occupy as much as 25%–40% of a rat's active period, but it is specifically elicited when an animal is suffering a stress-induced conflict or frustration. Grooming may also be playing a deactivating role in restoring behavioural homeostasis (Gispen and Isaacson, 1981).

A great variety of neuropeptides induce grooming: adrenocorticotropic hormone and related neuropeptides (Gispen et al.,

* Corresponding author. Tel.: +54 351 433 2021; fax: +54 351 433 2020.
E-mail address: mefisiol@mater.fcm.unc.edu.ar (M.E. Celis).

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1975), β-endorphin (Gispen et al., 1976) prolactin (Drago et al., 1980), α-melanocyte stimulating hormone (Torre and Celis, 1986), neuropeptide-EI (Sanchez et al., 1997), oxytocin and related neuropeptides (Drago et al., 1986; Caldwell et al., 1986). All these neuropeptides exert their action, at least partly, by facilitation of dopamine neurotransmission in the brain (Gispen and Isaacson, 1981; Isaacson et al., 1983). However, Sanchez et al. 2001, found another neurotransmitter, also related to the catecholaminergic system, involved with neuropeptide-EI. These authors specifically observed that the neuropeptide increases noradrenaline content in the nucleus accumbens (Sanchez et al., 2001). Based on these results, the present study was designed to establish the role of the noradrenergic system on the regulation of EGB and MA by neuropeptide-EI.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 250–300 g, were housed and cared for at the Laboratory of Physiology, School of Medicine, National University of Córdoba, Argentina, under the guidelines provided by the Institutional Animal Care of this same institution. The animals were kept under strictly controlled conditions of light (lights on 06:00–20:00 h) and temperature (21–23 °C), with *ad libitum* access to food and water.

2.2. Surgery and protocols

A stainless steel cannula (14 mm long, 0.65 mm o.d.) was implanted into the third ventricle of the animals, at the appropriate rostral/caudal coordinates (anteroposterior: 3.2 mm; lateral: 0 mm; vertical: 0.40 mm) according to the atlas by König and Klippel (1963). The cannula was cemented into place with dental acrylic. Behavioural testing began 7 days after surgery. i.c.v. injections (1 μl) were performed 15 min before the test.

Each rat was administered two injections with an interval of 5 min. The injections were as follows. Day 1: two administrations of artificial cerebral spinal fluid (aCSF) (control); Day 2: neuropeptide-EI (1 μg) followed by aCSF; Day 3: antagonist or agonist followed by aCSF; Day 4: first antagonist or agonist followed by neuropeptide-EI; Day 5: neuropeptide-EI (1 μg) followed by aCSF. The test on day 5 was undertaken to ascertain whether any tissue damage had been inflicted by the consecutive injections. If similar values were obtained on day 2 and day 5, we assumed that no apparent damage had been caused by the injection (data not shown).

2.3. Preparation of the drugs

All drugs, neuropeptide-EI (Bachem, Basel) and the different agonists and antagonists, were diluted in aCSF except for dobutamine which was diluted in ethanol (2% W/V). The following concentrations of antagonists were used: phentolamine, 4 and 8 μg/μl; propranolol, 0.25, 0.5 and 1 μg/μl; metoprolol, 0.25, 0.5 and 1 μg/μl; ((±)-1-[2,3-(Dihydro-7-methyl-1*H*-inden-4-yl) oxy]-3-[(1-methylethyl)amino]-2-butanol) (ICI 118.55), 40 and 80 ng/μl. The concentrations of agonists used were: isoproterenol, 7.5, 10 and 15 μg/μl; dobutamine, 6.25 and 12.5 μg/μl.

2.4. Post treatment behavioural analysis

After injection the rats were observed and scored as described (Gispen and Isaacson, 1981; Gispen et al., 1975; Celis and Torres, 1993). Tests were carried out between 9:00 to 14:00 at 22±2 °C in an isolated room, illuminated with an overhead fluorescent light. Rats were placed in cages with transparent walls (40×49×25 cm); EGB and MA were observed and scored every 15 s during 40 min (15 to 65 min after drug administration), for five consecutive days. The following features were used to determine each activity:

EGB: vibrating movements of forelegs, washing of forelegs and head, cleansing of hind legs, body, tail, genitals and scratching;

MA: locomotion (i.e., crossing the cage) and rearing (i.e., raising both forelegs from the ground, resting them, or not, on the cage wall).

2.5. Statistical analysis

One-way analysis of variance followed by Bonferroni post-hoc test for multiple comparisons was used for the statistical analysis of grooming behaviour and non parametric Kruskal Wallis test followed Dunns post-hoc test were used for the motor activity analysis. A P value ≤ 0.05 was considered a significant difference.

3. Results

3.1. Effect of a general α-adrenoceptor antagonist on neuropeptide-EI induced excessive grooming behaviour and motor activity

An i.c.v. injection of 1 μg neuropeptide-EI induced the characteristic EGB (Figs. 1–6) and MA (Table 1), in accordance

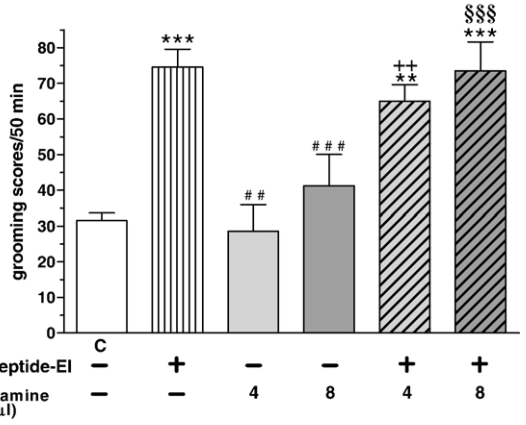

Fig. 1. Effects of intracerebroventricular administration of neuropeptide-EI (1 μg/μl) and phentolamine (4 μg/μl n=5 and 8 μg/μl n=4) on excessive grooming score of the behavioural test. Each bar represents the mean±S.E.M. *** P<0.001 and ** P<0.01 with respect to the control (C), ### P<0.001 and ## P<0.01 compared to neuropeptide-EI,++ P<0.01 with respect to the phentolamine 4 μg/μl and §§§ P<0.001 compared to phentolamine 8 μg/μl.

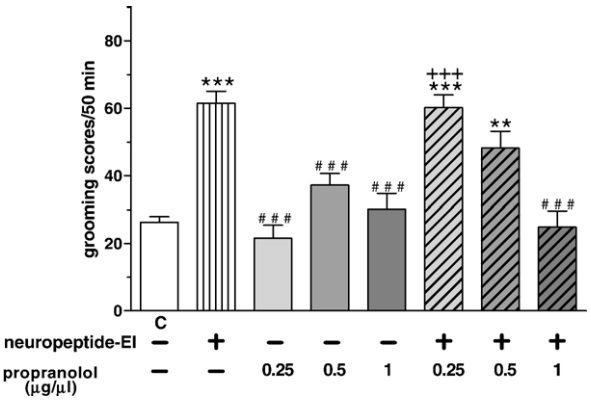

Fig. 2. Effects of intracerebroventricular administration of neuropeptide-EI (1 μg/μl) and propranolol (0.25 μg/μl, 0.5 μg/μl and 1 μg/μl $n=6$) on excessive grooming behaviour test. Each bar represents the mean±S.E.M. *** $P<0.001$ and ** $P<0.01$ compared to control (C), ## $P<0.01$ compared to neuropeptide-EI and++$P<0.001$ compared to propranolol 0.25 μg/μl.

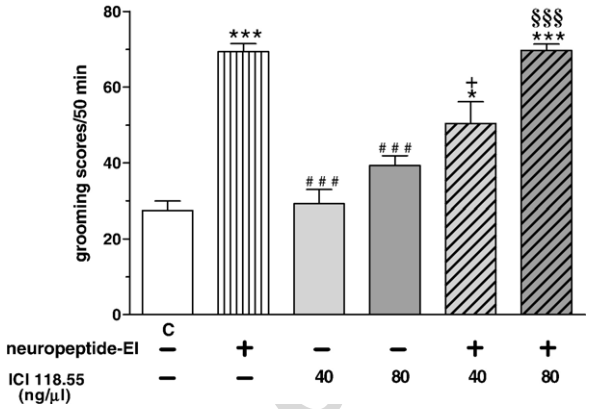

Fig. 4. Effects of intracerebroventricular administration of neuropeptide-EI (1 μg/μl) and ICI 118.551 (40 ng/μl $n=5$ and 80 ng/μl $n=7$) on excessive grooming behaviour test. Each bar represents the mean±S.E.M. *** $P<0.001$ and * $P<0.05$ with respect to control (C), ### $P<0.001$ compared to neuropeptide-EI,+ $P<0.05$ with respect to ICI 118.551 40 ng/μl and §§§ $P<0.001$ compared to ICI 118.551 80 ng/μl.

with our previous reports (Sanchez et al., 2001; Berberian et al., 2002). Regarding phentolamine, an α-adrenoceptor antagonist, none of the concentrations administered (4 and 8 μg/μl) elicited any modifications in the studied parameters. Administration of phentolamine 5 min before the neuropeptide-EI injection in no way modified this peptide's effect.

It is important to note that when neuropeptide-EI was re-administered at the end of all the experimental series, it still induced the same effects in the rats (data not shown).

3.2. Effect of a general β-adrenoceptor antagonist on neuropeptide-EI induced excessive grooming behaviour and motor activity

By itself, propranolol did not induce any changes on the studied parameters with respect to the control rats (0.25, 0.5 or 1 μg/μl doses). The administration of low concentrations of propranolol (0.25 and 0.5 μg/μl) 5 min before injecting neuropeptide-EI, did not modify the characteristic effect of the peptide. However, when a dose of 1 μg/μl was used, both the EGB and MA induced by the peptide was completely blocked (Fig. 2 and Table 1).

3.3. Effect of specific β-adrenoceptor antagonist on neuropeptide-EI induced excessive grooming behaviour and motor activity

With the object of studying which β receptor is involved in the aforementioned results, the effects of metoprolol a β_1-adrenoceptor antagonist, and ICI 118.55, a β_2-adrenoceptor antagonist, were tested.

We observed that metoprolol has a similar behaviour to propranolol (Fig. 3 and Table 1) when administered at equal doses. This β_1-adrenoceptor antagonist blocks the effects of neuropeptide-EI on EGB in a dose response manner when it is injected before the peptide, but it does not exert any influence when administered on itself.

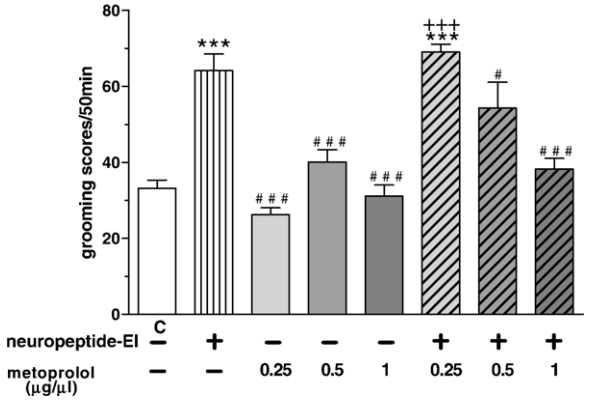

Fig. 3. Effects of intracerebroventricular administration of neuropeptide-EI (1 μg/μl) and metoprolol (0.25 μg/μl $n=5$, 0.5 μg/μl $n=7$ and 1 μg/μl $n=10$) on excessive grooming behaviour test. Each bar represents the mean±S.E.M. *** $P<0.001$ with respect to control (C), ### $P<0.001$ and ## $P<0.05$ with respect to neuropeptide-EI and++ $P<0.001$ with respect to metoprolol 0.25 μg/μl.

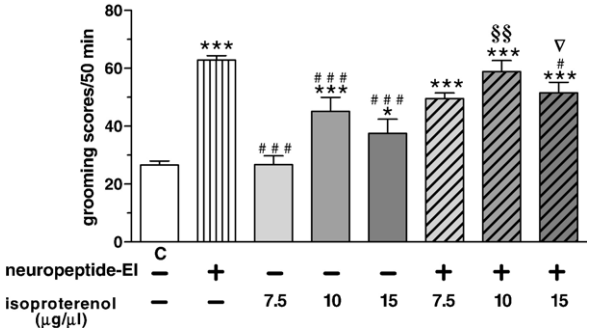

Fig. 5. Effects of intracerebroventricular administration of neuropeptide-EI (1 μg/μl) and isoproterenol (7.5 μg/μl $n=7$, 10 μg/μl $n=12$ and 15 μg/μl $n=8$) on excessive grooming behaviour test. Each bar represents the mean±S.E.M. *** $P<0.001$ and * $P<0.05$ with respect to control (C), ### $P<0.001$and # $P<0.05$ with respect to neuropeptide-EI,++ $P<0.001$ with respect to isoproterenol 7.5 μg/μl, §§ $P<0.01$ with respect to isoproterenol 10 μg/μl and ▽ $P<0.05$ with respect to isoproterenol 15 μg/μl.

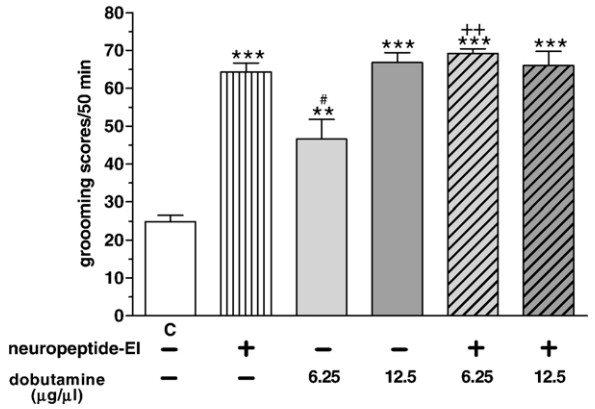

Fig. 6. Effects of intracerebroventricular administration of neuropeptide-EI (1 μg/μl) and dobutamine (6.25 μg/μl and 12.5 μg/μl $n=5$) on excessive grooming behaviour test. Each bar represents the mean±S.E.M. *** $P<0.001$ and ** $P<0.01$ with respect to control (C), # $P<0.05$ with respect to neuropeptide-EI and++ $P<0.01$ compared to dobutamine 6.25 μg/μl.

On the contrary, the β_2-adrenoceptor antagonist ICI 118,55 had no effect on neuropeptide-EI induced EGB (Fig. 4) and MA (data not shown) at any of the doses studied (40 and 80 ng/μl).

3.4. Effect of a general β-adrenoceptor agonist on neuropeptide-EI induced excessive grooming behaviour and motor activity

After the aforementioned results, isoproterenol, a general β-adrenoceptor agonist, was tested. Fig. 5 shows that although doses of 10 and 15 μg/μl isoproterenol increase EGB, this augmentation does not reach the degree induced by the peptide. When the agonist was administered before neuropeptide-EI at doses of 7.5 and 10 μg/μl, the effect of the neuropeptide was not affected. However, when a higher dose (15 μg/μl) of isoproterenol was administered previously it decreased neuropeptide-EI induced EGB (Fig. 5). Notwithstanding, isoproterenol was incapable of modifying MA (Table 1).

3.5. Effect of a β_1-adrenoceptor agonist, on neuropeptide-EI induced excessive grooming behaviour and motor activity

Dobutamine (6.25 and 12.5 μg/μl doses), increased EGB in a dose response manner. Nevertheless, when the agonist was injected 5 min before the peptide it did not modify the effect of neuropeptide-EI (Fig. 6). As observed with isoproterenol, dobutamine elicited no effect on MA (Table 1).

4. Discussion

Several studies have shown that neuropeptide-EI has a specific effect on the ventral tegmental area, activating the mesolimbic dopaminergic system; it also exerts an effect on noradrenergic pathways when inducing EGB and MA, as it modifies the levels of noradrenaline in the nucleus accumbens (Sanchez et al., 1997, 2001). For this reason, the present investigation was designed to study the role of the noradrenergic system on the induction of EGB and MA by neuropeptide-EI, and to determine whether this neurotransmitter is actually involved in the regulation of these behaviours.

Although the adrenergic system is scarcely represented in the area, and very diffuse in the central nervous system (Gehlert et al., 1993; Aston-Jones et al., 1995), it has acquired increasing importance in the modulation of processes that take place in the caudate putamen whose fibers are originated in the locus coeruleus (Wolfgang, 1998). Furthermore, anatomical and neurochemical studies have shown the existence of projections from the locus coeruleus to the striatum, and have found receptor sites for this neurotransmitter in the caudate putamen (Marien et al., 1994; Delfs et al., 1998).

Several studies suggest that β-adrenoceptors may be involved in the behavioural changes related to stress, and that the injection of propranolol could prevent these changes and simultaneously decrease serum levels of corticosterone; on the contrary, isoproterenol can induce the inverse behaviour (Zhang et al., 2001). Additionally, Angrini et al. observed that both propranolol and buspirone, a serotogenic agonist, have an

Table 1
Effects of intracerebroventricular administration of neuropeptide-EI (1 μg/μl) and different agonist or antagonist on motor activity (crossing the cage and rearing)

Control	NEI	Propranolol (μg/μl)			Propranolol (μg/μl)+NEI		
		0.25	0.5	1	0.25	0.5	1
5.0±3.5	7.5±3.5[a]	2.0±1.0[b]	4.0±2.5	2.5±2.5	6.5±8.5	7.5±7.8	0.0±2.5[b]
		Metoprolol (μg/μl)			Metoprolol (μg/μl)+NEI		
		0.25	0.5	1	0.25	0.5	1
		1.0±0.5[b]	3.0±3.5	0.5±2.8[b]	3.0±2.3	2.0±5.5	3.0±1.3[b]
		Isoproterenol (μg/μl)			Isoproterenol (μg/μl)+NEI		
		7.5	10	15	7.5	10	15
		14.0±6.5	4.5±2.8	9±9.3	19.0±11.5	4.5±1.5	9.5±6.8
		Dobutamine (μg/μl)			Dobutamine (μg/μl)+NEI		
		6.25		12.5	6.25		12.5
		3.0±1.8		7.0±4.0	5.0±4.0		6.0±5.8

In all experimental series the volume injected was 1 μl. Values represent the median±interquartilic range.
[a] $P<0.05$ with respect to control.
[b] $P<0.05$ with respect to neuropeptide-EI.

anxiolytic effect on peripheral movements (raising, cleaning, immobility and defecation) measured in the open-field test. These authors propose that the action of these β-adrenoceptor antagonists are possibly mediated by the same brain system (Angrini et al., 1998) and could be acting as sedatives. It is therefore possible to suggest that neuropeptide-EI acts via this system inducing a state of anxiety in the rat. This hypothesis was also proposed by other authors who observed that this peptide stimulates exploratory behaviour and increases anxiety in rats when injected into the ventromedial nucleus (Gonzalez et al., 1998).

The β-adrenergic mechanism appears to be critical due to the fact that propranolol is capable of blocking the effect of neuropeptide-EI on the studied behaviours. As this particular effect was the object of this study, we used two specific β-adrenoceptor antagonists for these receptors, metoprolol and ICI 118.55, a β_1- and a β_2-adrenoceptor antagonist respectively. Both propranolol and metoprolol had a similar effect, blocking the neuropeptide-EI induced behaviours. However, the β_2-adrenoceptor antagonist was not capable of modifying the induced EGB or MA. Futhermore, two β-adrenoceptor agonists, isoprotenol, a general β-adrenoceptor agonist, and dobutamine, a β_1-specific adrenoceptor agonist, elicited a similar response to neuropeptide-EI, strongly suggesting that noradrenergic stimulation is involved in these behaviours.

Comparing the results of the present study with those of other authors, it is important to note that β-adrenoceptors are involved in other behaviours such as sexual behaviour. For example, sexual behaviour is significantly stimulated in ovariectomized-adrenolectomized rats injected with estradiol benzoate and α-melanotropin or noradrenaline in the median eminence, and this effect is antagonized by the administration of propranolol and metoprolol prior to the α-melanotropin or noradrenaline treatment (Scimonelli et al., 2000).

We also analyzed the involvement of α-adrenoceptors using phentolamine. However, this drug evidenced no effect on the EGB and MA behaviours induced by neuropeptide-EI, indicating that the activity of α-adrenoceptors in this pathway is of minor importance.

Our results support the hypothesis that there is a relationship between neuropeptide-EI and the noradrenergic system and that, similarly to other endogenous peptides such as neurotensin, cholecystokinin, substance P and α-melanocyte stimulating hormone, this peptide could also be exerting some sort of modulating effect on the central nervous system. DOPAC/DA ratios indicate that dopamine plays a main role in neuropeptide-EI induced grooming (Sanchez et al., 2001). Hence, it is possible that the adrenergic receptor involved in the effect of neuropeptide-EI is a site regulating the activity of dopaminergic neurons, as final effectors of neuropeptide-EI's action.

In conclusion, this study evidences that the noradrenergic system is involved in EGB and MA behaviours induced by i.c.v. administration of neuropeptide-EI, and that this effect is mediated by β-adrenoceptors, more specifically β_1-adrenoceptors, based on the fact that metoprolol blocks the effect of neuropeptide-EI, and dobutamine, a β_1-adrenoceptor agonist, exerts a similar effect to the neuropeptide.

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