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

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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.,

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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

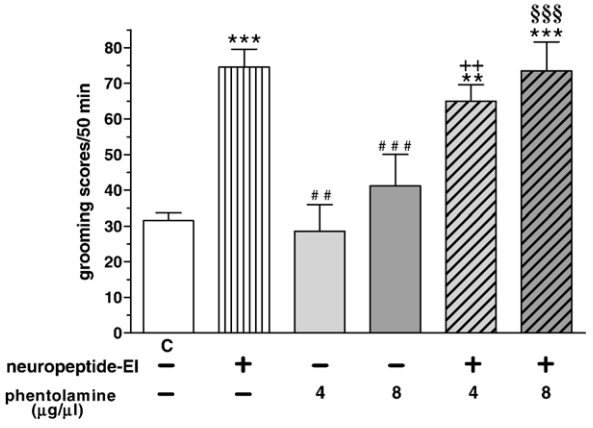

Fig. 1. Effects of intracerebroventricular administration of neuropeptide-EI (1 μg/μl) and phentolamine (4 μg/μl n=5 and 8 μg/μl n=4) on excessive grooming score of the behavioural test. Each bar represents the mean±S.E.M. *** P<0.001 and ** P<0.01 with respect to the control (C), ### P<0.001 and ##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