European Journal of Pharmacology 740 (2014) 97–102

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Correlation between algogenic effects of calcitonin-gene-related peptide (CGRP) and activation of trigeminal vascular system, in an *in vivo* experimental model of nitroglycerin-induced sensitization

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ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form
23 June 2014
Accepted 25 June 2014
Available online 10 July 2014

Keywords:
Calcitonin gene-related peptide
Nitroglycerin
Trigeminal vascular system
Migraine
Rat

Chemical compounds studied in this article:
Nitroglycerin (PubChem CID: CID 4510)

ABSTRACT

The neural mechanism(s) underlying migraine remain poorly defined at present; preclinical and clinical studies show an involvement of CGRP in this disorder. However current evidence pointed out that CGRP does not exert an algogenic action per se, but it is able to mediate migraine pain only if the trigeminal-vascular system is sensitized. The present study was addressed to investigate CGRP-evoked behavior in nitric oxide (NO) sensitized rats, using an experimental model of nitroglycerin induced sensitization of trigeminal system, looking at neuropeptide release from different cerebral areas after the intra-peritoneal (i.p.) administration of NO-donors. CGRP injected into the rat whisker pad did not induce significant changes in face rubbing behavior compared to controls. On the contrary, CGRP injected in animals pre-treated with 10 mg/kg nitroglycerin significantly increased the time spent in face rubbing. Nitroglycerin pre-treated animals did not show any rubbing behavior after locally injected saline. Furthermore, the i.p. treatment with nitroglycerin produced an increase of CGRP levels in brainstem and trigeminal ganglia, but not in the hypothalamus and hippocampus. The absolute amounts of CGRP produced in the brainstem were lower compared to those in the trigeminal ganglion; however, after nitroglycerin stimulation the percentage increase was higher in the brainstem. In conclusion, findings presented in this study suggest that CGRP induces a painful behavior in rats only after sensitization of trigeminal system; thus supporting the concept that a genetic as well as acquired predisposition to trigemino- vascular activation represents the neurobiological basis of CGRP nociceptive effects in migraineurs.

1. Introduction

Migraine is a common and debilitating disorder, occurring with a prevalence of 15–20% in the adult population. Migraine is characterized by recurrent attacks of head pain, which is usually unilateral and pulsating, moderate to severe in intensity, and lasting from 4 to 72 h (Gasparini et al., 2013; Goadsby et al., 2002; Headache Classification Committee of the International Headache Society, 2013). Although a full understanding of the neural mechanism(s) underlying migraine remains unknown, a convergence of findings from basic research and clinical trials suggests a pivotal role of calcitonin gene-related peptide (CGRP) in the patho-physiology of the disease (Hansen and Ashina, 2014).

CGRP is a 37aminoacid neuropeptide identified in the early 80's (Amara et al., 1982), widely expressed within the trigeminal pain pathway both at peripheral and central level (Hansen and Ashina, 2014). High concentrations of immunoreactive CGRP have been detected at the level of trigeminal nerve, where the peptide is localized in 50% of neurons (Walker and Hay, 2013) and is released after stimulation by capsaicin, KCl or electrical impulse (Capuano et al., 2007; Goadsby et al., 1988). Findings in support of CGRP involvement in migraine are the following: 1) increased CGRP levels have been found in serum, cerebrospinal fluid, and saliva of patients during migraine attacks (Ashina et al., 2000; Bellamy et al., 2006; Edvinsson and Goadsby, 1995; Goadsby et al., 1990; Juhasz et al., 2003); 2) the administration of triptans and CGRP inhibitors causes a reduction in circulating CGRP levels (Goadsby, 2006; Ho et al., 2008; Olesen et al., 2004); 3) the intravenous infusion of small doses of CGRP induces migraine in patients suffering from migraine with typical aura (Hansen et al., 2010).

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http://dx.doi.org/10.1016/j.ejphar.2014.06.046
0014-2999/

Interestingly, CGRP injected to non-migraineurs induces a sensation of head fullness, but not headache (Howden et al., 1988); likewise, the systemic administration of CGRP induces migraine-like attacks in migraineurs rather than in healthy controls (Hansen et al., 2010). These findings lead to postulate that CGRP might not have an algogenic action per se, but is able to induce typical migraine pain only under conditions of trigeminal vascular system sensitization. To test this hypothesis, in the present study we used an experimental model of trigeminal sensitization in the rat (Greco et al., 2008). Nitric oxide (NO) donors have been used to activate the trigeminal-vascular system (Messlinger et al., 2012); these authors showed that a close correlation exists between NO and CGRP in the generation of migraine pain. More recently, nitroglycerin was shown to increase spinal trigeminal activity, which was reversed by a CGRP receptor antagonist (Feistel et al., 2013). Within this framework, in the present study we have investigated CGRP-evoked pain related behavior in rats under basal conditions or after nitroglycerin-induced sensitization of the trigeminal system. Moreover, we also investigated CGRP production from different nervous system areas after intra-peritoneal (i.p.) administration of nitroglycerin or vehicle.

2. Material and methods

2.1. Drugs

Experiments were performed with a commercially available preparation of nitroglycerin (Nitroglicerina®, Bioindustria L.I.M., Novi Ligure Alessandria, Italy). This is a colorless sterile solution containing 5 mg/1.5 ml of nitroglycerin. CGRP was purchased from Tocris Bioscience (Bristol, UK). The neuropeptide was freshly dissolved in deionised water and further dilutions were made in sterile water.

2.2. Animals

Male Wistar rats aged 8–12 weeks (weight range 200–300 g) were used for *in vivo* experiments. Animals were obtained from the breeding facilities of Catholic University and were housed on a 12 h light–dark cycle at 22 ± 2 °C, with free access to food and drinking water. On the day of experiment, animals were acclimatized to the testing room for at least 2 h before testing. All animals were used only once and were sacrificed immediately after the experiments. This study was conducted according to the EEC Council Directive 86/609, and the guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). In addition, the study protocol was approved by Local Ethic Committee for Animal Care and Use of the Faculty of Medicine, Catholic University in Rome as well as by the Italian Ministry of Health (authorization to P. Navarra). All efforts were made to minimize animal suffering, and a preliminary sample size calculation was conducted to reduce the number of animals used.

2.3. Experimental design

1 μg of CGRP or corresponding volumes (50 μl) of vehicle (sterile injectable water) were injected subcutaneously into the right side of upper lip, in proximity to the nostril, using a 100 μl Hamilton syringe. After the injection, the rat was put in an observation cage consisting in a glass chamber ($30 \times 30 \times 30$ cm) with mirrored sides, and its behavior was video-recorded for 1 h. Videos were analyzed using JWatcher software (developed at Dan Blumstein's Lab University of California Los Angeles & The Animal Behavior Lab, Macquarie University, Sydney); recording time was divided in 3-min blocks and the number of seconds that the animals spend rubbing the injected area with the ipsilateral fore- or hind paw was recorded for each block. These experiments were carried out either under basal conditions or in rats pre-treated with 10 mg/kg nitroglycerin i.p., 4 and 24 h before the administration CGRP or vehicle into the whisker pad.

Another series of experiments was performed to investigate CGRP content in different areas of the nervous system following nitroglycerin injection at different time-points. A fixed dose of nitroglycerin (10 mg/kg) was administered in single-dose experiments by i.p. route. The animals were randomly distributed to two experimental groups (control and treatment) receiving nitroglycerin or vehicle (sterile injectable water) by the same route. Eight rats per experimental groups were sacrificed by decapitation 2 h, 4 h, and 24 h after drug injection. Trigeminal ganglion, brainstem, hypothalamus and hippocampus from the same rat were rapidly collected (Capuano et al., 2011; Tringali et al., 2012), placed in liquid nitrogen and subsequently stored at −80 °C until tissue homogenization. The latter was performed in fixed volumes

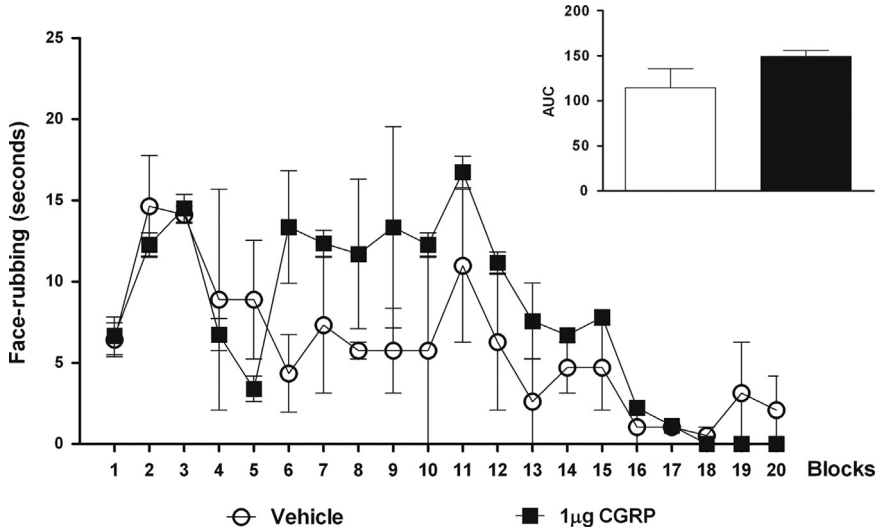

Fig. 1. CGRP locally injected into the rat whisker pad did not induce significant differences in face rubbing behavior compared to control. Recording time was divided in 3 min blocks and the number of seconds that animals spent on face rubbing was recorded for each block. Results are from two independent experiments, each including three replicates per experimental group. Data are expressed as face-rubbing seconds and area under the curves, AUC (insert). Means ± 1 S.E.M. of six replicates per group.