European Journal of Pharmacology 749 (2015) 32–38

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular pharmacology

Effects and underlying mechanisms of human opiorphin on cardiovascular activity in anesthetized rats

Xiao-zhu Tian [a,1], Yong Chen [a,1], Lu Bai [a], Pan Luo [a], Xue-jing Du [a], Qiang Chen [a], Xin-min Tian [b,*]

[a] Experimental Teaching Center of Biology, and Institute of Cell Biology, School of Life Sciences, Lanzhou University, 222 Tian Shui South Road, Lanzhou 730000, PR China
[b] Tropical Crops Genetic Resources Research Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou 571737, Hainan, PR China

ARTICLE INFO

Article history:
Received 13 February 2014
Received in revised form
16 November 2014
Accepted 18 November 2014
Available online 25 November 2014

Keywords:
Human opiorphin
Cardiovascular
Hypertension
Tachycardia
Sympathetic ganglia
Adrenal medulla

ABSTRACT

The present study was performed to investigate the peripheral cardiovascular effects of opiorphin in anesthetized rats. Intravenous (i.v.) injection of opiorphin (50–500 nmol/kg) caused marked dose-dependent increase in blood pressure and heart rate. The pressor and tachycardic responses induced by opiorphin (300 nmol/kg, i.v.) were significantly decreased by pretreatment with angiotensin-converting enzyme inhibitor captopril or angiotensin II type 1 (AT1) receptor antagonist valsartan, which suggested that endogenous angiotensin may be involved in the response to opiorphin. Pretreatment with α-adrenoreceptor antagonist phentolamine and β-adrenoceptor antagonist propranolol respectively attenuated the pressor response induced by opiorphin. Propranolol, but not phentolamine, inhibited the tachycardic response. Moreover, reserpine blocked both responses to opiorphin. These findings indicated that the effects of opiorphin to increase blood pressure and heart rate might be due to the stimulation of sympathetic ganglia. Additionally, studies with bilaterally adrenalectomized rats showed that adrenal medulla may be involved in the cardiovascular regulation of opiorphin. In addition, pretreatment with nonselective opioid receptor antagonist naloxone did not modify the cardiovascular responses to opiorphin, suggesting that the effects of opiorphin were not related to the opioid system. Furthermore, radioimmunoassay (RIA) showed that opiorphin significantly increased endogenous levels of angiotensin II and angiotensin III. In summary, all the results indicate that the cardiovascular effects induced by opiorphin are mediated through the renin–angiotensin system (RAS), the sympathetic ganglia and adrenal medulla, but not the opioid system.

1. Introduction

Wisner et al. (2006) isolated from human saliva a pentapeptide QRFSR, named opiorphin, encoded by human *PROL*1 gene. It has been also reported that opiorphin inhibits two zinc metal ectopeptidases, human neutral ecto-endopeptidase NEP (EC 3.4.24.11) and human ecto-aminopeptidase APN (EC 3.4.11.2). NEP and APN are membrane-bound metallopeptidases that play important roles in turning off neural and hormonal peptide signaling at the cell surface by hydrolyzing a variety of neuropeptides. NEPs are widely distributed in tissues (Dussaule et al., 1993; Ronco et al., 1988), particularly in the heart, kidney and lungs where they have important functions as ectoenzymes, catalyzing the postsecretory processing or metabolism of several signaling peptides, some decrease blood pressure, such as atrial natriuretic factor (ANF), kinins, neurotensin, enkephalins, Substance P, and endorphins, whereas others may increase blood pressure, such as angiotensin, endothelin, and perhaps vasopressin (Erdös and Skidgel, 1989). APNs, which are located in the intestinal, lung and kidney epithelial cells (Kenny and Maroux, 1982; Matsas et al., 1985; Semenza, 1986), cleave the N-terminals of biologically active peptides such as enkephalin, angiotensin, neurokinin and cytokines, and exert profound activity on vital processes such as immune response, cellular growth, and blood pressure control. Given the cardiovascular effects of these peptides, peptidase inhibitors could play important roles in regulating the cardiovascular system, which are yet to be studied.

Owning to the physiological importance and the critical role of NEP and ANP in metabolism of neuropeptides and hormonal signals, dual peptidase inhibitors, which simultaneously block NEP and APN activities have been studied for several years. Non-peptide dual inhibitors, kelatorphan, RB-101 and RB3007 completely inhibit NEP and APN in vivo to produce antinociceptive, antidepressant, and anxiolytic effects in rodents. (Jackson and

* Corresponding author. Tel./fax: +86 898 23301850.
E-mail address: tianxm333333@gmail.com (X.-m. Tian).
[1] The authors who contributed equally to this study.

http://dx.doi.org/10.1016/j.ejphar.2014.11.025
0014-2999/

Mi, 2008; Leguen et al., 2003). Similar to these dual inhibitors, opiorphin, acting as an inhibitor of both NEP and APN, has been implicated in some biological actions, including analgesic activity, colonic contraction and modulation of penile erection (Tian et al., 2009; Tong et al., 2008; Ueda et al., 2000). Rougeot et al. (2003) characterized sialorphin, a peptide mediator synthesized predominantly in the submandibular gland of rat, and suggested that rat sialorphin is an endocrine peptide signal whose secretion is stimulated under adrenergic-mediated response to environmental stress in male rat. In addition, opiorphin and sialorphin are expressed in saliva and corporal tissue, and released into blood stream in the regulation of epinephrine (Rougeot et al., 2003; Tong et al., 2006). It was also reported that sialorphin improved erectile function and blood pressure (Tong et al., 2008). Recently, our group reported that opiorphin increased blood pressure of conscious rats through the renin–angiotensin system (RAS) by elevating endogenous angiotensin II levels (Fang et al., 2014). These data suggested that opiorphin may produce hypertension by inhibiting the metabolism of endogenous angiotensin in conscious rats, but the cardiovascular effects of opiorphin in anesthetized rats and the detailed mechanisms are still unknown.

To further evaluate the pharmacological characterization of opiorphin, in the present study, we investigated the cardiovascular effects of opiorphin at peripheral level in anesthetized rats and the underlying mechanisms involved in these responses.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley (S-D) rats (250 ± 30 g) were obtained from the Experimental Animal Center of Lanzhou University. All animals were cared for and experiments were carried out in accordance with the European Community guidelines for the use of experimental animals (86/609/EEC) (Fang et al., 2009). All the protocols in this study were approved by the Ethics Committee of Lanzhou University, China.

2.2. Drugs

Opiorphin was synthesized by a solid phase peptide synthesis method and purified by high-performance liquid chromatography (HPLC). Mass spectrometric analysis showed purity exceeded 98.5%. Drugs used and their sources in parentheses were: captopril (Hubei Teyer Pharmaceutical Co., Ltd.); valsartan (Scenery chemistry limited liability company of Hefei, China); reserpine injection (The first affiliated hospital of Lanzhou University); propranolol hydrochloride and phentolamine hydrochloride from Sigma Chemical Company (USA); naloxone hydrochloride were obtained from Fluka. All the drugs were dissolved in normal saline (NS), and the solutions were divided into aliquots and stored in 2 ml plastic tubes at -20 °C. The aliquots were thawed and used on the day of the experiment. During an experiment, the agonist solutions were kept on crushed ice. Radioimmunoassay (RIA) kits were obtained from Beijing North Institute of Biological Technology.

2.3. Measurement of cardiovascular responses

Experiments were performed as described in previous report (Fang et al., 2009). Rats were anesthetized by intraperitoneal (i.p.) injection of urethane (1.2 g/kg) and supplemental doses of urethane were administered as needed to maintain a uniform level of anesthesia. Polyethylene 50 catheters (Beckton Dickinson) were inserted into the left external jugular vein for i.v. administration of drugs. The right carotid artery was cannulated with polyethylene catheter and linked to a pressure transducer (YT-100) with its output connected to a recorder system (model BL-420F, Taimeng Technology Corporation of Chengdu, China). The animals were then allowed to recover for 40 min. The mean arterial pressure (MAP) and heart rate (HR) were measured directly from the pre-calibrated BL-420F recorder system.

2.4. Experimental protocol

A syringe was used for i.v. administration. Dose–response curves were obtained for the effect of $50-500$ nmol/kg of human opiorphin on MAP and HR. It was administered from lower to higher doses by rapid i.v. injection in a volume of 200 μl and an interval of at least 30 min was allowed between each injection. Reproducible results were obtained with three injections of 300 nmol/kg of opiorphin at 30 min intervals, indicating that desensitization does not occur to the pressor response induced by opiorphin. Therefore, the next dose was administered after return of MAP and HR to baseline levels with an interval of at least 30 min. The dose of opiorphin (300 nmol/kg) were tested individually in 50 independent rats and each rat received only one dose of peptide as a control and then we investigated the effect of each antagonist on the cardiovascular responses induced by opiorphin in each of the 6–10 rats. As previous reports (Cachofeiro et al., 1992; Fang et al., 2009; Liu et al., 2008), captopril (10 mg/kg), valsartan (10 mg/kg), phentolamine (10 mg/kg), propranolol (2 mg/kg) or naloxone (2 mg/kg) was injected i.v. 15 min prior to opiorphin. Reserpine (2 mg/kg) was injected i.p. 24 h prior to opiorphin (Kong et al., 2008). We recorded the value 15 min after administration of each antagonist to compare with the control in order to evaluate the respective effect of each antagonist on the effect induced by opiorphin. According to the previous report (Kong et al., 2008), under urethane anesthesia, the two adrenal glands were exposed, and removed after ligation of the vessels perfusing the gland. Adrenalectomized rats received NS to drink and were used for experiments 48 h after surgery. Studies for each dose of the agonist alone and for each dose of the agonist plus antagonist were conducted in separate groups of rats.

2.5. RIA

To further investigate the underlying mechanisms involved in the cardiovascular response of opiorphin, the levels of plasma hormones were determined by RIA (Batt et al., 1990). 2 ml blood sample was collected from right carotid artery 1 min after administration of opiorphin (300 nmol/kg, i.v.). A 2 ml blood sample was collected from the right carotid artery 1 min after administration of opiorphin (300 nmol/kg, i.v.). The quantitative in vitro measurement of angiotensin II, Norepinephrine (NE), angiotensin III, vasopressin and aldosterone were carried out by using RIA kit according to the manufacturer's instructions.

2.6. Statistical analysis

Data were expressed as means $\pm$ S.E.M. Responses were analyzed with a one-way ANOVA followed by Dunnett's post-hoc test. Differences between the groups were analyzed by paired Student's t test; A probability level of less than 0.05 ($P < 0.05$) was considered to be statistically significant.

3. Results

3.1. Effects of i.v. administration of opiorphin on MAP and HR in anesthetized rats

In the experiment with opiorphin, baseline MAP and HR values in anesthetized rats were 108.62 ± 2.98 mmHg and 388.80 ± 13.20 bpm