Peptides 47 (2013) 1–6

Contents lists available at ScienceDirect

Peptides

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

Intermedin enhances sympathetic outflow via receptor-mediated cAMP/PKA signaling pathway in nucleus tractus solitarii of rats

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ARTICLE INFO

Article history:
Received 20 March 2013
Received in revised form 7 May 2013
Accepted 7 May 2013
Available online 29 June 2013

Keywords:
Intermedin
Sympathetic outflow
Protein kinase A
Nucleus tractus solitarii

ABSTRACT

Direct administration of intermedin (IMD) into the brain elicits cardiovascular effects different from the systemic administration. Nucleus tractus solitarii (NTS) is an important region for the cardiovascular regulation. The present study was designed to determine the effect of IMD on modulating the sympathetic outflow and its related molecular mechanism in the NTS. Renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP) were recorded in anesthetized rats. Site-specific microinjection of IMD (20 pmol) bilaterally into the NTS significantly increased RSNA and MAP. IMD-evoked increases of RSNA and MAP were almost abolished by pretreatment with receptor antagonist ADM22-52, an adenylyl cyclase (AC) inhibitor SQ22536, or a protein kinase A (PKA) inhibitor Rp-cAMP. However, pretreatment with another receptor antagonist calcitonin gene-related peptide (CGRP)8-37 did not suppress the increases of RSNA and MAP induced by IMD. Furthermore, IMD increased the cyclic adenosine monophosphate (cAMP) level, which was inhibited by ADM22-52 pretreatment in the NTS. These results suggest that IMD participates in the sympathetic nerve activity and central regulation of the cardiovascular system and a receptor-mediated cAMP/PKA signaling pathway is involved in IMD-induced effects in the NTS.

1. Introduction

Intermedin (IMD) belongs to the calcitonin gene-related peptide (CGRP) superfamily, which consists of adrenomedullin (AM), CGRP, calcitonin and amylin [5,25,26,28]. IMD is a multifunctional peptide with a wide distribution, not only in mammalian peripheral tissues, but also in the central nervous system (CNS) [12,19,25]. In mammals, IMD was also a potent, circulating vasodilatory peptide, because intravenous or intraperitoneal administration of IMD causes hypotension [23,28]. However, IMD applied either intracerebroventricularly (icv) or directly into the nucleus tractus solitarius (NTS) had a hypertensive effect [7,10,29]. These results suggest that the existence of distinct regulatory mechanisms between the central and peripheral control of the cardiovascular system by IMD.

IMD shows its biological activities mainly through the G protein-coupled calcitonin receptor-like receptor (CRLR), which is shared by CGRP and AM [25,29]. Three different subtypes of the receptor-activity modifying protein (RAMP) determine ligand selectivity of CRLR. The complex of CRLR and RAMP1 shows a higher affinity with CGRP; the complex of CRLR and RAMP2 or RAMP3 has a higher interaction with AM [4,17]. However, IMD can interact with the three complexes non-selectively [25]. These receptor components for IMD are abundant in both central and peripheral tissues involved in the cardiovascular regulation.

In mammals, the NTS integrates autonomic visceral inputs from peripheral organs with afferent signals arising from the higher CNS and plays an important role in the cardiovascular reflexes. Furthermore, the peripheral baroreceptor, chemoreceptor and cardiopulmonary afferents make their first synapse in the medial subnucleus of the NTS (mNTS) [1,9,31]. Because PAMP-2, CRLR and IMD are expressed abundantly in the NTS [12,20,21] and IMD microinjection into NTS increases blood pressure (BP) [7], we hypothesize that NTS is an important site on which IMD acts

Abbreviations: IMD, intermedin; NTS, nucleus tractus solitarii; RSNA, renal sympathetic nerve activity; MAP, mean arterial pressure; HR, heart rate; SNA, sympathetic nerve activity; BP, blood pressure; AC, adenylyl cyclase; PKA, protein kinase A; CGRP, calcitonin gene-related peptide; cAMP, cyclic adenosine monophosphate; AM, adrenomedullin; icv, intracerebroventricularly; CRLR, calcitonin receptor-like receptor; RAMP, receptor-activity modifying protein; CNS, central nervous system; mNTS, medial subnucleus of the nucleus tractus solitarii.

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http://dx.doi.org/10.1016/j.peptides.2013.05.002

directly to modulate sympathetic nerve activity (SNA). However, whether the IMD modulates SNA and its downstream signaling pathway involved in the sympathetic activation is not well elucidated.

The present study was designed to determine the role of the IMD in modulating sympathetic outflow and cardiovascular system function in the NTS and its related signaling pathway.

2. Materials and methods

2.1. Animals

Experiments were carried out on male Sprague-Dawley rats weighing between 300 and 350 g. All animals were housed under controlled conditions with a 12:12-h light–dark cycle. Food and water were available to the animals ad libitum. The experiments were performed according to the Experimental Animal Care and Use Committee of Nanjing Medical University and complied with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996). All efforts were made to minimize the number of animals used and their suffering. The rat was anesthetized with urethane (800 mg/kg, i.p.) and α-chloralose (40 mg/kg, i.p.). Supplemental doses of anesthetic agents were administered intravenously during the experiment for maintaining an appropriate level of anesthesia. The right carotid artery of rat was cannulated for continuous recording of MAP [36].

2.2. Recording of renal sympathetic nerve activity (RSNA)

RSNA used to evaluate the dynamic changes of sympathetic outflow was recorded as previously reported [37]. The left renal sympathetic nerve was isolated from a retroperitoneal incision. The nerve was cut distally to exclude its afferent activity. The RSNA was amplified with an AC/DC differential amplifier (Model 3000; A-M System, Washington, DC, USA) with a low-frequency cutoff at 60 Hz and a high-frequency cutoff at 3000 Hz, and integrated at a time constant of 0.1 s. The raw RSNA, integrated RSNA and MAP were simultaneously recorded through a PowerLab data acquisition system (8SP; AD Instruments, Bella Vista, NSW, Australia) [33].

2.3. Microinjection into the mNTS

The coordinates for the mNTS microinjection, taken from the rat brain atlas (Paxinos G and Watson C, 2005), were: 0.4–0.5 mm rostral to the calamus scriptorius, 0.5–0.6 mm lateral to the midline and 0.4–0.5 mm ventral to the dorsal surface of the medulla. The bilateral mNTS microinjections were carried out with two glass micropipettes (about 50 μm tip diameter) and finished within 1 min (50 nl for each side of the mNTS). Before experiments began, the functional identification of NTS site is through a depressor response of at least 25 mmHg caused by L-glutamate (2 nmol) microinjection [32]. After the experiments finished, 50 nl of 2% Evans blue was injected into the injection sites for marking. Then the brains were removed and placed in 10% formalin, and sectioned to verify the microinjection sites according to the atlas of Paxinos and Watson. The microinjection sites used for data analysis were within the marginal regions of the NTS.

2.4. Measurement of cyclic adenosine monophosphate (cAMP) level in mNTS

The rats were euthanized with an overdose of pentobarbital (100 mg/kg, iv). The brains were removed and quickly frozen with liquid nitrogen and stored at −80 °C until being sectioned. Coronal sections were made using a cryostat microtome (Leica CM1900-1-1, Wetzlar, Hessen, Germany) to obtain the mNTS. The mNTS areas were punched out with a 15-gauge needle (inner diameter 1.5 mm) and put in the lysis buffer for homogenizing. The cAMP level of the mNTS was determined by ELISA method using an enzyme immunoassay kit from Cayman Chemical Company (Ann Arbor, MI48108, USA) [15].

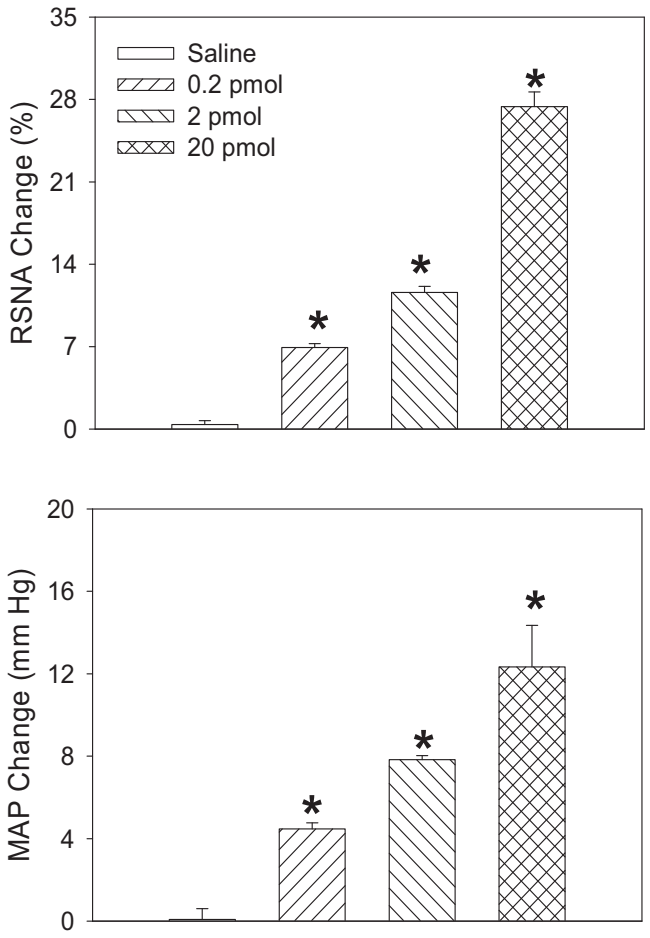

Fig. 1. Dose effects of saline or three doses of IMD (0.2, 2 and 20 pmol) microinjection into the mNTS on RSNA and MAP. Values are mean ± SE. $^{*}P < 0.05$ vs. saline. $n = 6$ for each group. Abbreviation: RSNA, renal sympathetic nerve activity; MAP, mean arterial pressure.

2.5. Chemicals

Rat intermedin (IMD), human CGRP8-37 (CGRP receptor antagonist, it can block the interreaction between IMD and CRLR/RAMP1 complex) and human ADM22-52 (AM receptor antagonist, it can block the interreaction between IMD and CRLR/RAMP2 or CRLR/RAMP3 complex) were from Bachem AG (Hauptstrasse, Bubendorf, Switzerland); 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ22536, an adenylyl cyclase inhibitor) and rp-adenosine-3′,5′-cyclic monophosphothionate (Rp-cAMP, a protein kinase A inhibitor) were purchased from Sigma Chemical Co (St. Louis, MO, USA). The chemicals were dissolved in saline.

2.6. Experimental protocols

The rats were randomly divided into each group ($n = 6$ for each). The mNTS microinjections were carried out for administration of saline, three doses of IMD (0.2, 2, 20 pmol), CGRP8-37 (60 pmol), human ADM22-52 (375 pmol), SQ22536 (2 nmol), Rp-cAMP (1 nmol) or IMD (20 pmol) pretreated with human CGRP8-37