Biochemical Pharmacology 88 (2014) 75–85

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Activation of PI3K/Akt signaling in rostral ventrolateral medulla impairs brain stem cardiovascular regulation that underpins circulatory depression during mevinphos intoxication

Ching-Yi Tsai [1], Alice Y.W. Chang [1], Julie Y.H. Chan, Samuel H.H. Chan *

Center for Translational Research in Biomedical Sciences, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung 83301, Taiwan, ROC

ARTICLE INFO

Article history:
Received 26 November 2013
Accepted 13 January 2014
Available online 22 January 2014

Keywords:
Mevinphos intoxication
PI3K/Akt/NF-κB signaling
NOS II/peroxynitrite cascade
Rostral ventrolateral medulla
Impairment of brain stem cardiovascular regulation

ABSTRACT

As the most widely used pesticides in the globe, the organophosphate compounds are understandably linked with the highest incidence of suicidal poisoning. Whereas the elicited toxicity is often associated with circulatory depression, the underlying mechanisms require further delineation. Employing the pesticide mevinphos as our experimental tool, we evaluated the hypothesis that transcriptional upregulation of nitric oxide synthase II (NOS II) by NF-κB on activation of the PI3 K/Akt cascade in the rostral ventrolateral medulla (RVLM), the brain stem site that maintains blood pressure and sympathetic vasomotor tone, underpins the circulatory depressive effects of organophosphate poisons. Microinjection of mevinphos (10 nmol) bilaterally into the RVLM of anesthetized Sprague-Dawley rats induced a progressive hypotension that was accompanied sequentially by an increase (Phase I) and a decrease (Phase II) of an experimental index for the baroreflex-mediated sympathetic vasomotor tone. There were also progressive augmentations in PI3K or Akt enzyme activity and phosphorylation of p85 or Akt(Thr308) subunit in the RVLM that were causally related to an increase in NF-κB transcription activity and elevation in NOS II or peroxynitrite expression. Loss-of-function manipulations of PI3K or Akt in the RVLM significantly antagonized the reduced baroreflex-mediated sympathetic vasomotor tone and hypotension during Phase II mevinphos intoxication, and blunted the increase in NF-κB/NOS II/peroxynitrite signaling. We conclude that activation of the PI3K/Akt cascade, leading to upregulation of NF-κB/NOS II/peroxynitrite signaling in the RVLM, elicits impairment of brain stem cardiovascular regulation that underpins circulatory depression during mevinphos intoxication.

1. Introduction

It has been reported [1] that pesticide poisoning is the single most common means for suicidal purposes in the world. Understandably, as the most widely used pesticides in the globe, the highest incidence of suicidal poisoning is associated with the organophosphate compounds [2]. The clinical presentations of organophosphate poisons, which include salivation, seizure, hypotension, coma and death [3], are generally believed to entail over-stimulation of peripheral and central synapses by the accumulated acetylcholine that result from cholinesterase inhibition [4,5]. Detailed cellular and molecular mechanisms that underlie organophosphate poisoning [6], particularly the elicited circulatory depressive effects, require further delineation.

In our search for the cellular and molecular underpinnings of circulatory depression during organophosphate poisoning, our laboratory has concentrated on mevinphos (3-[dimethoxyphosphinyl-oxyl]-2-butenoic acid methyl ester), a US Environmental Protection Agency Toxicity Category I pesticide, the most commonly used organophosphate for suicidal purposes in Taiwan [7]. Earlier work from our laboratory [8] demonstrated that mevinphos elicits cardiovascular toxicity by acting on the rostral ventrolateral medulla (RVLM), a brain stem site known classically for maintaining arterial pressure (AP) and sympathetic vasomotor tone [9]. Based on spectral analysis of AP signals, we found that mevinphos induces an increase (Phase I) followed by a decrease

Abbreviations: aCSF, artificial cerebrospinal fluid; Akt, serine/threonine protein kinase; EMSA, electrophoretic mobility shift assay; HR, heart rate; LF, low-frequency; 3-MA, 3-methyladenine; NF-κB, nuclear factor-κB; NO, nitric oxide; NOS, nitric oxide synthase; NRS, normal rabbit serum; PIP_3, phosphatidylinositol-3,4,5-triphosphate; PIP_2, phosphatidylinositol-4,5-bisphosphate; PI3K, phosphoinositide 3-kinase; PH, pleckstrin homology; PKB, protein kinase B; PKG, protein kinase G; PTEN, phosphatase and tensin homologue deleted on chromosome ten; RVLM, rostral ventrolateral medulla; SHR, spontaneously hypertensive rats; TNF-α, tumor necrosis factor-α.

* Corresponding author.
E-mail address: shhchan@cgmh.org.tw (Samuel H.H. Chan).
[1] These authors contributed equally to this study.

0006-2952/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bcp.2014.01.014

(Phase II) in the power density of the low-frequency (LF) component, which emanates from the RVLM and serves as an index for brain stem cardiovascular regulation in the form of baroreflex-mediated sympathetic vasomotor tone [10]. Mechanistically, we have established [11–13] that nitric oxide (NO) generated by NO synthase I (NOS I) in the RVLM, followed by activation of the soluble guanylyl cyclase/cGMP/protein kinase G (PKG) cascade, is responsible for enhancing baroreflex-mediated sympathetic vasomotor tone and AP during Phase I mevinphos intoxication [14–16]. On the other hand, peroxynitrite formed by a reaction between superoxide anion and NOS II-derived NO, which is upregulated transcriptionally by nuclear factor-κB (NF-κB), in the RVLM underlies the impairment of brain stem cardiovascular regulation that leads to hypotension during Phase II [13,17].

The phosphoinositide 3-kinase (PI3K)/serine/threonine protein kinase (Akt) signaling, which is well-established to be involved in tumorigenesis [18,19], is now known to be involved in a host of regulatory functions, including cellular proliferation, migration, cell cycle progression, survival and apoptosis [20,21]. PI3Ks are classified into three groups according to their structure, substrate specificity and distinct roles in cellular functions [22–25]. Class I PI3K is the only group that generates phosphatidylinositol-3,4,5-triphosphate (PIP_3) by phosphorylating phosphatidylinositol-4,5-bisphosphate (PIP_2). The most common form of class I PI3K is a heterodimer made up of a catalytic subunit (p110) and a regulatory subunit (p85). Akt, also referred to as protein kinase B (PKB), is a serine/threonine kinase. Mammals have three closely related Akt genes, encoding the isoforms Akt1 (PKBα), Akt2 (PKBβ), and Akt3 (PKBγ). All Akt isoforms consist of a N-terminal pleckstrin homology (PH) domain that preferentially binds PIP_3, a kinase domain and a C-terminal regulatory tail [26,27]. Two specific sites, one in the kinase domain (Thr308 in Akt1) and the other in the C-terminal regulatory region (Ser473 in Akt1), have to be phosphorylated for full Akt activation. As a downstream target of PI3K [28,29], Akt is activated by ligation of PIP_3 and subsequent phosphorylation of Thr308 and Ser473 by 3′-phosphoinositide-dependent kinase 1 and 2 at the membrane.

Several lines of evidence suggest that the PI3K/Akt cascade in the RVLM may participate in brain stem cardiovascular regulation [30,31]. Furthermore, PI3K/Akt signaling participates in the induction of NOS II expression [32], and class IA PI3K is essential for the dimerization of NOS II [33]. There are also indications that PI3K/Akt signaling induces NOS II gene expression through NF-κB activation [34,35]. It follows that the PI3K/Akt cascade in the RVLM may act as signals upstream to NF-κB/NOS II activation in the elicitation of impairment of brain stem cardiovascular regulation that leads to circulatory depression during mevinphos intoxication. The present study validated this guiding hypothesis.

2. Materials and methods

2.1. Ethics statement

All experimental procedures carried out in this study were approved by the Institutional Animal Care and Use Committee of the Kaohsiung Chang Gung Memorial Hospital, and were in compliance with the guidelines for animal care and use set forth by that committee. All efforts were made to reduce the number of animals used and to minimize animal suffering during the experiment.

2.2. Animals

Specific pathogen-free adult, male Sprague-Dawley rats (236–337 g; n = 358) purchased from the Experimental Animal Center of the National Science Council or BioLASCO, Taiwan, were used. They were housed in an AAALAC International-accredited Center for Laboratory Animals under temperature control (24–25 °C) and 12-h light–dark cycle. Standard laboratory rat chow and tap water were available ad libitum.

2.3. General preparation

Under an initial pentobarbital sodium anesthesia (50 mg kg^{-1}, i.p.), animals received preparatory surgery that included intubation of the trachea and cannulation of a femoral artery and vein. During the recording session, anesthesia was maintained by intravenous infusion of propofol (Zeneca, Macclesfield, UK) at 20–25 mg kg^{-1} h^{-1}. We have demonstrated [36] that this scheme provided satisfactory anesthetic maintenance while preserving the capacity of brain stem cardiovascular regulation. The head of the animal was thereafter fixed to a stereotaxic head holder (Kopf, Tujunga, CA, USA), and the body temperature was maintained at 37 °C with a heating pad. During the recording session, animals were allowed to breathe spontaneously with room air.

2.4. Mevinphos intoxication

We demonstrated in our initial study [8] that on systemic administration, mevinphos acts on the RVLM to elicit cardiovascular responses that are comparable to those elicited by microinjection of this organophosphate directly into the RVLM. The latter route of administration was therefore employed in the present study to produce site-specific mevinphos-induced cardiovascular actions [8,11,13]. AP signals recorded from the femoral artery were subject to on-line power spectral analysis [8,11,13]. We were particularly interest in the LF (0.25–0.8 Hz) component in the AP spectrum, which takes origin from the RVLM [37] and reflects the prevalence of baroreflex-mediated sympathetic neurogenic vasomotor tone that emanates from this brain stem site [10]. Heart rate (HR) was derived instantaneously from the AP signals. The AP spectrum and power density of the LF components were displayed continuously during the experiment, alongside pulsatile AP, mean AP (MAP) and HR, in a real-time manner.

2.5. Microinjection of test agents into the RVLM

Microinjection bilaterally of test agents into the RVLM, at a volume of 50 nL, was carried out with a stereotaxically positioned glass micropipette that was connected to a 0.5-μl Hamilton microsyringe (Reno, NV, USA). The coordinates used were 4.5–5 mm posterior to the lambda, 1.8–2.1 mm lateral to midline, and 8.1–8.4 mm below the dorsal surface of cerebellum [8,11]. These coordinates were selected to cover the ventrolateral medulla at which functionally identified sympathetic premotor neurons reside [9]. As shown previously [12], microinjected test agents exhibited a discrete diffusion of approximately 800 μm × 800 μm to an area in the medulla oblongata that is below the nucleus ambiguus, lateral to the inferior olivary nucleus and medial to the spinal trigeminal nucleus [38].

Test agents used in this study included mevinphos (kindly provided by Sinon Corporation, Taichung Country, Taiwan); PI3K inhibitors, LY294002 [39] (Promega, Madison, WI, USA) or wortmannin [40] (Calbiochem, San Diego, CA, USA); class I PI3K inhibitor, PI103 [41] (Calbiochem); class III PI3K inhibitor, 3-methyladenine (3-MA) [42] (Sigma–Aldrich, St. Louis, MO, USA); Akt inhibitor, API-2 [43] (Calbiochem); or antisera against phospho-p85 (Cell Signaling, Beverly, MA, USA) or phospho-Akt(Thr308) (Cell Signaling). Possible volume effect of microinjection was controlled by injecting the same amount of artificial cerebrospinal fluid (aCSF), normal rabbit serum (NRS) (Sigma–Aldrich) or solvent (1% DMSO for LY294002, API-2 or 3-MA; 2% DMSO for wortmannin or PI103). As in our previous study [12], 0.02% Triton X-100 (Sigma–Aldrich) was added to facilitate transport of the antiserum or NRS across the cell