Thiamethoxam induced hepatic energy changes in silver catfish via impairment of the phosphoryl transfer network pathway: Toxicological effects on energetics homeostasis


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ABSTRACT

Precise coupling of spatially separated intracellular adenosine triphosphate (ATP)-producing and ATP-consuming processes exerts a pivotal role in bioenergetic homeostasis of living organisms, and the phosphoryl transfer network pathway, catalyzed by adenylate kinase (AK) and pyruvate kinase (PK), is fundamental in cellular and tissue energetic homeostasis. Measurement of the phosphoryl transfer network can provide new information for understanding the alterations in hepatic energetic metabolism during exposition to insecticides, such as thiamethoxam. Therefore, the aim of this study was to evaluate whether exposition to thiamethoxam negatively affects the hepatic enzymes of the phosphoryl transfer network in silver catfish (Rhamdia quelen). Hepatic AK and PK activities were inhibited at 3.75 μg L⁻¹ after 24 h of exposure and at 1.125 and 3.75 μg L⁻¹ after 96 h of exposure compared with the control group. The hepatic ATP levels were decreased following 3.75 μg L⁻¹ thiamethoxam treatment after 24 h of exposure and at 1.125 and 3.75 μg L⁻¹ after 96 h of exposure compared with the control group. The enzymatic activity of the phosphoryl transfer network and ATP levels did not recover after 48 h of recovery in clean water. Thus, the inhibition of hepatic AK and PK activities by thiamethoxam caused impairment of energy homeostasis in liver tissue, decreasing hepatic ATP availability. Moreover, the absence of a mutual compensatory mechanism between these enzymes directly contributes to ATP depletion and to a severe energetic dysregulation, which may contribute to toxic effects caused by thiamethoxam.

1. Introduction

Although pesticides play a major role in increasing agricultural productivity, their abusive and indiscriminate use represents a major environmental and public health problem worldwide (Jallow et al., 2017), since only 0.1% of pesticides reach their target, and the remainder results in contamination of the ecosystem (Carriger et al., 2006). According to Joseph and Raj (2011), the presence of fish in practically all aquatic environments inevitably exposed them to a several of toxicologically and structurally different pesticides, including thiamethoxam.

Thiamethoxam 3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro) amine is a second-generation neonicotinoid insecticide that belongs to the thianicotinyl subclass and is widely used for the control of a vast spectrum of target insect pests (Rumbos et al., 2018). It exerts an antagonistic effect on the insect synaptic and extrasynaptic nicotinic acetylcholine (nACh) receptor (Thany et al., 2007). Although recent evidence has suggested that its high target specificity provides relatively low risk for non-target organisms (e.g., mammals) and the environment (Jeschke et al., 2011), some evidence have demonstrated the toxic effects of thiamethoxam for fishes, since this pesticide presents high water solubility (4 g L⁻¹). In this regard, the liver is the primary target of thiamethoxam-induced toxicity because hepatic tissue plays a principal role in thiamethoxam metabolism, and is metabolized into three major metabolites (CGA330050, CGA265307, and CGA223704), which mediate thiamethoxam-induced hepatotoxicity by oxidative stress in zebrafish (Danio rerio) (Yan et al., 2016) and histopathological lesions in pacamã (Lophiusolurus alexandri) (Albinati et al., 2017). However, the effects of fish contamination on hepatic tissue and energy homeostasis remain poorly understood. Thus,

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The concentration of thiamethoxam was measured in the water from detection (HPLC-DAD) using a column Synergi 4 Fusion RP 250 × 4.6 mm, 4 μm, for experimental purposes from a fish farm located in Rio Grande do Sul state (Brazil). The animals were transported alive and maintained for acclimation in 250 L fiberglass tanks with continuous aeration and controlled water parameters (temperature: 20.9 ± 0.3 °C, pH: 6.7 ± 0.03 and dissolved oxygen: 5.88 ± 0.42 mg L⁻¹) during one week. The animals were fed to apparent satiation with commercial pellets once a day and feeding continued during the experimental period. Any uneaten food, feces and other residues were removed daily 60 min after feeding.

The fish were allocated to 100 L tanks with continuous aeration and exposed for 0 and 96 h to thiamethoxam, and allowed 48 h of post-recovery in water without insecticide (54 fish; three replicates per concentration; six fish per replicate): 0.0 (control), 1.125, and 3.75 μg L⁻¹. Water quality variables (temperature, dissolved oxygen, alkalinity, hardness, ammonia, non-ionized ammonia and nitrite) were evaluated daily, and remained without alterations during all experimental period, and were measured following the methodologies recently published in details by Salbego et al. (2014). All procedures were approved by the Ethical and Animal Welfare Committee of the Universidade Federal de Santa Maria (protocol 067/2014).

2. Material and methods

2.1. Chemical

Thiamethoxam (250 mg kg⁻¹) was purchased in a Brazilian market as commercial name of Actara® (Syngenta). The insecticide was added to the test water at 1.125 and 3.75 μg L⁻¹ prior to the beginning of the tests, since this concentration is used in irrigated rice (Teló et al., 2001). Neumann et al. (2003) demonstrated that a spatially arranged intracellular enzymatic network catalyzed by AK and PK activities improved nucleotide exchange, facilitating communication between sites of ATP generation and ATP utilization (Saupe et al., 2000), exerting an essential role in the maintenance of energetic homeostasis. The enzyme AK facilitates intracellular communication and metabolic monitoring by amplification of metabolic signals by catalyzing the reaction ATP + AMP ↔ 2 ADP (Dzeja and Terzic, 2009). Moreover, PK is a key enzyme of the glycolytic route that catalyzes the irreversible transphosphorylation of phosphoenolpyruvate (PEP) to ADP to form pyruvate and ATP (Wang et al., 2002), with a fundamental role in the glycolytic pathway, which is the main route that provides energy to ensure proper liver tissue function. Dere et al. (2008) demonstrated that PK activity is inhibited in the liver, kidney, brain, and small intestine of rats exposed to the herbicide dinotro-o-cresol and the insecticide dichlorvos, impairing energetic metabolism. Thus, our hypothesis is that thiamethoxam can inhibit hepatic enzymes of the phosphotransfer network, impairing energetic cellular homeostasis.

Based on this evidence, the aim of this study was to evaluate whether exposure to thiamethoxam negatively affects the hepatic enzymes of the phosphotransfer network in silver catfish (Rhamdu quelen).

2.2. Fish maintenance and experimental design

Juvenile R. quelen (106.27 ± 20.21 g; 23.5 ± 1.19 cm) were collected for experimental purposes from a fish farm located in Rio Grande do Sul state (Brazil). The animals were transported alive and maintained for acclimation in 250 L fiberglass tanks with continuous aeration and controlled water parameters (temperature: 20.9 ± 0.3 °C, pH: 6.7 ± 0.03 and dissolved oxygen: 5.88 ± 0.42 mg L⁻¹) during one week. The animals were fed to apparent satiation with commercial pellets once a day and feeding continued during the experimental period. Any uneaten food, feces and other residues were removed daily 60 min after feeding.

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2.3. Sample collection and tissue homogenization

After 24 and 96 h of exposition, and at the end of experiment (48 h post-recovery), two animals from each tank (six fish per treatment at each given time, totaling 54 animals) were anesthetized with 50 mg L⁻¹ eugenol (Cunha et al., 2010). Thereafter, the hepatic tissue was removed and prepared to evaluate the activities of enzymes belonging to phosphotransfer network, as well as the hepatic ATP levels.

The hepatic tissue was washed and homogenized (1:10 w/v) in sucrose buffer (0.32 M sucrose, 1 mM ethylene glycol tetraacetic acid, and 10 mM Tris-HCl, pH 7.4) with a Potter-Elvehjem glass homogenizer. The homogenate was centrifuged (800 × g for 10 min at 4 °C) and an aliquot of the supernatant was utilized to evaluate AK activity. The pellet was rejected and the rest of the supernatant was centrifuged (10,000 × g for 15 min at 4 °C), and this supernatant was collected to measure PK activity.

2.4. Hepatic AK and PK activities

Hepatic AK activity was measured with a coupled enzyme assay with hexokinase (HK) and glucose 6-phosphate dehydrogenase (G6PD), according to Dzeja et al. (1999), and published in detail by Baldissera et al. (2017). The activity was expressed in pmol ATP formed per min per mg of protein.

Hepatic PK activity was assayed according the protocol established by Leong et al. (1981), and published in details by Baldissera et al. (2017). The activity was expressed in μmol pyruvate formed per min per mg of protein.

2.5. Hepatic ATP levels

The ATP levels in hepatic samples were measured using the Firefly Luciferase ATP assay kit (Beyotime®, China), following the manufacturer’s instructions. Result was expressed in pmol per mg of protein.

2.6. Protein content

Hepatic protein content was measured using the method described by Lowry et al. (1951), that use bovine serum albumin as a standard.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Thiamethoxam</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>1.125 3.75 96 h</td>
</tr>
<tr>
<td></td>
<td>1.34 ± 0.01 3.57 ± 0.03</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± standard error of thiamethoxam in water. Limit of detection: 0.06 μg L⁻¹; limit of quantification: 0.20 μg L⁻¹.

Note: n.d. (not detected).
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