Toxicon 150 (2018) 297–303

Contents lists available at ScienceDirect

Toxicon

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

Dipteran toxicity assays for determining the oral insecticidal activity of venoms and toxins

Shaodong Guo[1], Volker Herzig[*,1], Glenn F. King[*]

Institute for Molecular Bioscience, The University of Queensland, St. Lucia QLD 4072, Australia

ARTICLE INFO

Keywords:
Spider
Venom peptide
Toxin
Oral toxicity
Orally-active
Screening
Dipteran
Phyletic specificity

ABSTRACT

The growing world population is placing an increasing demand on food production. In addition, abuse and misuse of chemical insecticides has led to the evolution of resistance in insect pests as well as environmental damage. Together, these developments have created a demand for new insecticidal compounds to facilitate global food production. Arachnid venom peptides provide an environmentally-friendly alternative as potential bioinsecticides given their advantages of being fully biodegradable, highly potent, and phyletically selective. However, the use of arachnid venom peptides as bioinsecticides has been questioned due to their presumed lack of oral toxicity. Thus, the aim of this work was to develop screens for oral insecticidal activity. Based on the high susceptibility of dipterans to venom peptides, fruit flies (*Drosophila melanogaster*) and sheep blowflies (*Lucilia cuprina*) were selected for screening 56 arachnid venoms. 71.4% of these venoms caused 50% or higher mortality in *Drosophila*, whereas 30.4% were lethal to blowflies at oral doses of 1 or 30 μg/fly, respectively. We used these assays to compare the oral and injection activity of four well-known spider venom peptides (Hv1a, Hv1c, Dc1a and Ta1a). Hv1c and Ta1a only showed weak or no oral activity in both species, while Hv1a and Dc1a showed higher oral activity in blowflies than *Drosophila*. Overall, we have established screens for oral toxicity in two dipteran insects. Our results indicate that oral insecticidal activity is more widespread in arachnid venoms than expected, and that some arachnid venoms and venom peptides exhibit phyletic differences in oral toxicity.

1. Introduction

The world population has increased continually from 0.35 billion in the 14th century to a current estimate of 7.6 billion, placing increased demands on food production. Despite the advent of chemical insecticides to combat insect pests, which are the major cause of crop losses, approximately 10–14% of world crops are currently destroyed by insects (Oerke, 2006; Dhaliwal et al., 2010; Bebber et al., 2013; Herzig et al., 2014). Insect pests also vector a variety of human and livestock pathogens. For example, malaria, which is transmitted by mosquitoes, led to 730,500 human deaths in 2015 (Wang, 2016).

Despite the introduction of transgenic crops, chemical insecticides remain the dominant approach for controlling insect pests. An important problem for insecticides is the development of resistance in pest insects. Although numerous chemical insecticides have been developed, they are directed against very few molecular targets (King and Hardy, 2013), with > 75% of all insecticides targeting acetylcholinesterase, voltage-gated sodium channels, acetylcholine receptors, or GABA/glutamate-gated chloride channels (Casida, 2009). Other common disadvantages of organic chemical insecticides include poor selectivity and issues with biodegradability, which can have negative implications for the environment (Aktar et al., 2009). Together, these issues have led to a rapid decline of available insecticides and we are therefore faced with the challenge of fighting an increasing insect pest problem with fewer active insecticides (King and Hardy, 2013).

Spiders are the most successful venomous insect predators. They subdue prey by injecting venom into the body of insects, allowing the constituent venom peptides to target mainly ion channels in the insect nervous system (King and Hardy, 2013). Insecticidal peptides from spider venoms are promising bioinsecticides because they are highly potent, fully biodegradable, and have a high specificity resulting in a reduction of unwanted effects in off-target species (King and Hardy, 2013). To date, 466 insecticidal peptides have been isolated from spider venoms (Herzig et al., 2011; Pineda et al., 2017). Previous studies have demonstrated the potential of spider-venom peptides as bioinsecticides (Rohou et al., 2007; Hernandez-Campuzano et al., 2009; Windley et al., 2012; Konno et al., 2016). However, a major problem of spider venom peptides as bioinsecticides remains their presumed lack of oral activity (Nentwig, 1993).

* Corresponding authors.
E-mail addresses: v.herzig@uq.edu.au (V. Herzig), glenn.king@imb.uq.edu.au (G.F. King).
[1] Indicates joint first authors.

https://doi.org/10.1016/j.toxicon.2018.06.077
Received 30 April 2018; Received in revised form 7 June 2018; Accepted 15 June 2018
Available online 18 June 2018
0041-0101/ © 2018 Elsevier Ltd. All rights reserved.

Since spiders inject venom directly into prey, their venom peptides are not under any evolutionary selection pressure to enable them to cross the insect midgut in order to reach their molecular targets in the insect nervous system. Nevertheless, the insecticidal peptide ω-hexatoxin-Hv1a (Hv1a) from the spider *Hadronyche versuta* (Fletcher et al., 1997) exhibits oral activity against larvae of the lepidopterans *Helicoverpa armigera* and *Spodoptera littoralis* as well as ticks (Khan et al., 2006; Mukherjee et al., 2006). Venom-peptide OAIP1 from the Australian tarantula *Selenotypus plumipes* is also orally active, although it is 100-fold less potent orally than when administered by injection into cotton bollworms (*H. armigera*) (Hardy et al., 2013). However, even this level of oral potency could still be sufficient for use as a bioinsecticide as the oral LD_{50} of OAIP1 in *H. armigera* (104.2 pmol/g) is still > 460-fold lower than the oral LD_{50} of DDT (48.3 nmol/g) in *H. armigera* (McCaffery et al., 1991; Khan et al., 2006; Herzig and King, 2015). The fact that some insecticidal spider-venom peptides exhibit oral insecticidal activity could indicate that oral toxicity is more common than previously anticipated for spider-venom peptides. Unfortunately, there have been no systematic efforts to screen for orally-active insecticidal compounds from spider venoms, which is partially attributable to the lack of appropriate assays and the fact that screens for oral activity require larger amounts of venom compared to injection assays, which can be a limiting factor for small arachnids.

Thus, we have developed sensitive screens for oral toxicity using two species of dipterans, as insecticidal spider venom peptides are known to be particularly potent against dipterans (Windley et al., 2012). The dipteran species we used for these assays are sheep blowflies (*Lucilia cuprina*, mean weight 20–25 mg), which are a significant pest of livestock in Australia (Ikonomopoulou et al., 2016), and the smaller fruit flies (*Drosophila melanogaster*, mean weight 0.8–1.0 mg). Here we provide a detailed description of how to perform these assays and discuss their potential advantages and disadvantages.

2. Material and methods

2.1. Venoms and venom peptides

Venoms were collected by one of the authors (VH) by using either small electrical stimulations (for spider venoms, see details in (Herzig and Hodgson, 2008)) or by aggravating scorpions to sting onto a parafilm membrane from which venom was subsequently collected using a pipette. After collection, all venoms were dried by lyophilisation or by using a vacuum centrifuge, then they were reconstituted in water prior to experiments. Recombinant venom peptides were produced using a periplasmic expression system as previously described (Bende et al., 2014; Undheim et al., 2015) except for Hv1a, which was kindly supplied by Vestaron Corporation (Kalamazoo, MI, USA).

2.2. Oral toxicity assay in L. cuprina

We used a previously described method to determine toxicity by injection in sheep blowflies (*Lucilia cuprina*) (Bende et al., 2013) and modified it to allow for oral administration of venoms and venom compounds.

Step 1: Obtaining L. *cuprina* larvae

Sheep blowfly larvae were placed in 250 ml plastic containers with vermiculite as bedding material and maintained at room temperature (21–23 °C) in the dark. Under these conditions it takes about 10 days for the larvae to develop into adult blowflies.

Step 2: Starvation

After eclosure ("hatching"), no food or water was provided for 6–12 h prior to experiments. Blowflies were then placed on dry ice for a few minutes until all flies stopped moving. Each blowfly was individually transferred into a 2 ml tube with a small hole in the lid. Blowflies housed in tubes were then placed at room temperature for 10–15 min to recover from the cooling. After transferring all flies into 2 ml tubes, any inactive or dead blowflies were removed before commencing toxicity assays.

Step 3: Oral toxicity assay

Venom peptides were dissolved in 5% sucrose solution and 3 µl of the venom peptide-sucrose solution was applied to the inside of a 2 ml tube containing a single blowfly. Flies were kept in these tubes overnight at room temperature without additional food or water. Thereafter, flies were transferred (by cooling down on dry ice) into 250 ml plastic boxes containing vermiculite as bedding material (to absorb any spilled water), a sugar cube, and a piece of wet cotton wool placed into the lid of a 50 ml Falcon tube. Flies that received the same treatment were placed in the same box. The behaviour of flies (dead, paralysed or alive) was assessed at regular time intervals after applying venom peptide-sucrose solution (e.g., 1, 2, 4, 24, 48 and 72 h).

Step 4: Weight determination and calculation of dose-response curves

Before transferring blowflies from 2 ml tubes to 250 ml plastic boxes, the average fly-weight per group (usually in groups of $n = 10$ that received the same treatment) was determined. This was achieved by cooling blowflies on dry-ice until they stopped moving, then quickly transferring them into a pre-weighed 250 ml plastic container. To obtain dose-response curves, we typically used 6–10 different doses with three groups ("repeats") of $n = 10$ flies per dose. For all doses from each repeat, a separate dose-response curve was calculated and the final PD_{50} (= dose that causes 50% paralysis) and LD_{50} (= dose that causes 50% lethality) values were calculated as a mean ± standard error of the mean (SEM) of the three repeats. All dose-response curves were fitted with Prism 7.0 (GraphPad Software, Inc., San Diego, CA, USA) using a sigmoidal dose-response with a variable slope according to the following equation:

$$Y = \frac{100}{1 + 10^{(\mathrm{LogDose}_{50} - X)*nH}}$$

where Y is the percentage of affected (i.e. paralysed or dead) flies, X is the logarithm of the venom/venom peptide dose (in mg of venom/venom peptide per g of fly), and nH is the Hill coefficient (i.e., variable slope factor). Before calculating the dose-response curves, the mortality in the treatment groups were adjusted by the mortality of the control group by using the Henderson-Tilton formula:

$$Corrected\,(\%) = \left(1 - \frac{n\ in\ C\ before\ treatment * n\ in\ T\ after\ treatment}{n\ in\ C\ after\ treatment * n\ in\ T\ before\ treatment}\right) * 100$$

where n is the number of paralysed/dead flies, T refers to the "treatment group", and C refers to the "control group".

2.3. Oral toxicity assay in D. melanogaster

Step 1: *Drosophila* culture

Wild-type *Drosophila melanogaster* (Canton S strain) were cultured in 25 × 95 mm polypropylene culture vials (Genesee Scientific, California, USA) filled with 1/5th of *Drosophila* diet on the bottom (the composition of the fly diet is indicated in the Supplementary Material). The *Drosophila* culture was kept at room temperature (21–23 °C) using a 12/12 h day/night cycle. In order to prevent overcrowding, a maximum of 100 *Drosophila* were used per vial. Mating occurs very quickly, starting from 1 day after hatching, and it takes about 10 days at room

Download English Version:

https://daneshyari.com/en/article/8394203

Download Persian Version:

https://daneshyari.com/article/8394203

Daneshyari.com