MINI-REVIEW

Entomotoxicity of jaburetox: revisiting the neurotoxic mechanisms in insects

Yuri Correia Barreto[1], Maria Eduarda Rosa[1], Ana Paula Zanatta[1], Bruna Trindade Borges[1], Stephen Hyslop[2], Lúcia Helena Vinadé[1], Chárіston André Dal Belo[1]*

[1]Centro Interdisciplinar de Pesquisa em Biotecnologia (CIPBiotec), Universidade Federal do Pampa (UNIPAMPA), Avenida Antônio Trilha 1847, Campus São Gabriel, São Gabriel 97300-000, RS, Brazil

[2]Departamento de Farmacologia, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), Rua Tessália Vieira de Camargo, 126, Cidade Universitária Zeferino Vaz, 13083-887, Campinas, SP, Brazil

***Correspondence to:** Chárіston André Dal Belo, E-mail: charistonbelo@unipampa.edu.br, Tel/Fax: +55 55 3237 0850

Received: 29 May 2020 | **Revised:** 04 August 2020 | **Accepted:** 31 August 2020 | **Published:** 15 September 2020

J Venom Res (2020), Vol 10, in press

Note: This is not the final version of this article, which will be available in the near future.

ABSTRACT

Ureases are metalloenzymes that hydrolyze urea to ammonia and carbamate. The main urease isoforms present in the seeds of *Canavalia ensiformis* (jack bean urease – JBU and canatoxin) exert a variety of biological activities. The insecticidal activity of JBU is mediated, at least in part, by jaburetox (Jbtx), a recombinant peptide derived from the JBU amino acid sequence. In this article, we review the neurotoxicity of Jbtx in insects. The insecticidal activity of Jbtx has been investigated in a variety of insect orders and species, including Blattodea (the cockroaches *Blatella germânica*, *Nauphoeta cinerea*, *Periplaneta americana* e *Phoetalia pallida*), Bruchidae (*Callosobruchus maculatus* – cowpea weevil), Diptera (*Aedes aegypti* – mosquito), Hemiptera (*Dysdercus peruvianus* – cotton stainer bug; *Oncopeltus fasciatus* – large milkweed bug, and the kissing bugs *Rhodnius prolixus* and *Triatoma infestans*), Lepidoptera (*Spodoptera frugiperda* – fall army worm) and Orthoptera (*Locusta migratoria* – locust). In *N. cinerea*, the injection of Jbtx induces marked alteration of locomotor and grooming behavior, whereas in *T. infestans* Jbtx causes leg paralysis, an extension of the proboscis and abnormal antennal movements. Electromyographical analysis showed that Jbtx causes complete neuromuscular blockade in *P. pallida*. The same treatment in *N. cinerea* and *L. migratoria* causes a decrease in the action potential firing rate. Jbtx forms membrane pore-channels compatible with cations in bilipid membranes. A study using *B. germanica* voltage-gated sodium (Nav1.1) channels that were heterologously expressed in *Xenopus laevis* oocytes correlated the entomotoxicity of Jbtx with the activation of these channels. Taken together, these findings demonstrate the potential of this peptide as a natural pesticide.

KEYWORDS: Behavioral alterations, *Canavalia ensiformis,* entomotoxicity, jack bean urease, neuromuscular blockade, plant ureases, voltage-gated sodium channel

INTRODUCTION

Ureases (urea amidohydrolase, EC 3.5.1.5) are metalloenzymes belonging to the superfamily of amidohydrolases and phosphotriesterases and are widely distributed in plants, fungi and bacteria. Most ureases contain two Ni^{2+} ions in their active site (Follmer et al, 2002; Krajewska, 2009; Carter et al, 2011; Zambelli et al, 2011) and catalyze the hydrolysis of urea to ammonia and carbamate, with the latter subsequently decomposing into ammonia and carbon dioxide (Callahan et al, 2005; Carter et al, 2009). The urease extracted from *C. ensiformis* seeds (jack bean urease – JBU) has been studied for approximately 100 years and was initially crystallized by James B Sumner, who demonstrated the protein nature of this enzyme (Sumner, 1926) and was subsequently awarded the Nobel Prize in Chemistry (1946) for this work.

The presence of nickel ions in the active site of the enzyme and their obligatory presence for catalytic activity was demonstrated ~50 years later, in 1975 (Dixon et al, 1975).

JBURE-I is the most abundant isoform of JBU present in *C. ensiformis* seeds and consists of 840 amino acids, with a molecular mass of 90,77kDa; the native protein consists of homogeneous monopolymers arranged into 540kDa hexamers (Figure 1A) (Sirko and Brodzik, 2000; Krajewska, 2009; Ligabue-Braun et al, 2013). Detailed information on the structural organization of ureases is provided by Ligabue-Braun et al (2013). In addition to JBU, *C. ensiformis* has two other isoforms of urease: canatoxin (a 95kDa dimer) (Carlini and Guimarães, 1981) and JBURE-II (a 78kDa polypeptide) (Mulinari et al, 2011).

The existence of biological activities unrelated to the ureolytic function of ureases was initially demonstrated by studies of the neurotoxicity of this protein in rodents and insects. Canatoxin, a highly toxic protein that produces convulsions when injected intraperitoneally in rats and mice, was isolated from jack beans in the early 1980s (Carlini and Guimarães, 1981). In addition to its neurotoxicity in mammals, canatoxin has insecticidal activity against insects such as *C. maculatus* (Bruchidae; cowpea weevil) and *R. prolixus* (Hemiptera; kissing bug). Canatoxin is cleaved by the enzyme cathepsin to yield a 10kDa peptide known as pepcanatox. This peptide may also be responsible for the lethality of canatoxin in insects that possess cathepsin-like digestive enzymes, whereas insects with trypsin-like digestive enzymes are not susceptible (Carlini et al, 1997). A new peptide equivalent to pepcanatox was identified when the N-terminal portion of the peptide obtained by the cleavage of canatoxin was aligned with the sequence of JBURE-II, a JBU isoform (Figure 1B). A cDNA fragment was amplified from this template and subcloned into an expression vector in *Escherichia coli* to produce the 91 amino acid recombinant peptide (~10kDa) referred to as jack bean urease toxin (jaburetox-2Ec or jaburetox, which lacks the fused V5-antigen present in the 2Ec version – Jbtx; Figure 1C) (Mulinari et al, 2007; Postal et al, 2012).

Numerous studies of *C. ensiformis* ureases and peptides over the past four decades have identified several biological activities associated with these molecules, including membrane rupture and permeabilization, pro-inflammatory and fungicidal properties and neurotoxicity in vertebrates and invertebrates (Carlini and Ligabue-Braun, 2016; Kappaun et al, 2018; Sá et al, 2020). In this article, we will focus on the physiological and behavioral dysfunctions caused by Jbtx in insects and the complex neuromodulatory mechanisms involved (Martinelli et al, 2014; dos Santos et al, 2019). The selectivity of this toxin towards insects means that this molecule potentially can be used to develop transgenic plants that are resistant to pests and disease (Kappaun et al, 2018).

GENERAL MECHANISMS OF NEUROTOXIC PESTICIDES

As agricultural pests, insects can cause extensive damage to food crops, resulting in substantial economic losses (Costa et al, 2008). Insects can also serve as reservoirs for various pathogens involved in debilitating human diseases (Ngai and McDowell, 2017). In developing countries such as Brazil, medically important insects are the cause of annual outbreaks of diseases such as dengue, chikungunya and zikavirus (Zara et al, 2016). Many broad-spectrum chemical pesticides act on the central and peripheral nervous systems of insects and humans to affect specific targets. For example, organophosphates and carbamates selectively inhibit the enzyme acetylcholinesterase (AChE). Other pesticides, such as pyrethroids, which are widely used as agricultural and domestic insecticides and for the topical treatment of scabies and lice, as well as mosquito repellent (Costa et al, 2008), bind to and delay the inactivation of voltage-gated sodium channels, leading to a stable hyperexcitable state. The insecticidal activity of neonicotinoids, such as imidacloprid, is attributed to the activation of nicotinic acetylcholine receptors (nAChR) where these substances mimic the neurotransmitter acetylcholine (ACh). However, unlike ACh, these compounds are not susceptible to enzymatic hydrolysis by AChE and their continuous activation of nAChR can lead to hyperexcitation, causing loss of muscle coordination, seizures and death from respiratory failure in vertebrates (Costa et al, 2008; Islam and Malik, 2018). The similarity of the neurochemical processes among many target and non-target species (including humans) means that pesticides can exert acute and chronic neurotoxicity in non-target species, including the stimulation of neurodegenerative diseases. For this reason, there is an urgent need to develop novel, environmentally friendly insecticides with greater selectivity for the insect nervous system (Franco et al, 2010; Ngai and McDowell, 2017; Islam and Malik, 2018).

LETHALITY OF JBTX

Mulinari et al (2007) examined the lethality of Jbtx in cotton stainer bugs (*D. peruvianus*) that were fed artificial seeds containing Jbtx (0.01%, w/w); lethality was time-dependent with 100% mortality occurring after 11 days, compared to insects fed with canatoxin (same dose), for which 20% of the insects were still alive at the end of the experiment. The insecticidal activity of Jbtx was also tested against *S. frugiperda* (fall army worm) in which digestion is based on trypsin-like activity and an alkaline intestine. After being fed leaf discs containing Jbtx (16.3µg every two days) the average weight of the larvae treated with Jbtx was ~30% less than the controls on the second day. All larvae died by the sixth day of treatment, after ingesting a total of 47µg of Jbtx (Mulinari et al, 2007). Martinelli et al (2014) examined the lethality of Jbtx in insects that were fed the peptide (*R. prolixus*) or injected with it (*R. prolixus* and *O. fasciatus*). In *R. prolixus*, a dose of 0.05µg/mg of body weight caused 100% mortality 48hr after injection, while in *O. fasciatus* that received

0.015µg/mg of body weight, the mortality was 80% in 96hr. In *R. prolixus* nymphs fed saline containing the peptide (final dose: 0.1µg/mg of body weight), the mortality was 80% after 24hr (Martinelli et al, 2014). Tomazetto et al (2007) showed that the intrathoracic injection of Jbtx (0.02-0.1µg/gm of body weight) was lethal in nymphs and adults of *T. infestans* (kissing bug), with 100% mortality ≥20hr after injection. Galvani et al (2015) also studied the effects of Jbtx on adult *T. infestans* and reported findings similar to those of Tomazetto et al (2007).

BEHAVIORAL ALTERATIONS

In *N. cinerea* cockroaches, the injection of Jbtx (8, 16 and 32µg/gm body weight) caused a 50% increase in the leg and antennal grooming activity compared to saline controls (dos Santos et al, 2019). Galvani et al (2015) also observed behavioral changes in *T. infestans* injected with the peptide, including limb paralysis, an extension of the proboscis and abnormal movements of the antennae; the latter involved bending the antenna upwards and executing irregular movements in the vertical plane. In *N. cinerea*, Jbtx (16 and 32µg/gm) markedly reduced the total distance travelled and increased the duration of stationary periods when cockroaches were not in movement, while the frequency of immobile episodes was affected only by a dose of 32µg/gm (dos Santos et al, 2019).

ELECTROMYOGRAPHIC ANALYSIS

Several studies have shown that Jbtx causes neuromuscular blockade in insects. Martinelli et al (2014) reported that Jbtx (32µg/gm) produced a time-dependent blockade of muscle twitch-tension responses in metathoracic coxal-abductor nerve-muscle preparations of *Phoetalia pallida* cockroaches, with complete neuromuscular paralysis occurring within 35 min. In a similar investigation, dos Santos et al (2019) observed that Jbtx caused a progressive reduction in the twitch amplitude of *N. cinerea* cockroach muscle that was maximal after 110min (reductions of 81% and 76% with 8µg/gm and 16µg/gm, respectively). In *L. migratoria* tarsal muscle preparations, Jbtx (2.5×10^{-7}µg/gm) reduced the amplitude of muscle action potentials by ~20% and that of nerve action potentials by 20-30% (dos Santos et al, 2019). Jbtx also caused a dose- and time-dependent reduction in the amplitude of spontaneous neural compound action potentials in *N. cinerea* isolated limb preparations (Zanatta et al, unpublished data).

NEUROPHYSIOLOGICAL AND BIOCHEMICAL ALTERATIONS

JBU and Jbtx (10^{-16}M and 10^{-15}M, respectively) exert an antidiuretic effect *in vitro* in Malpighian tubules isolated from *R. prolixus*. The antidiuretic effect of Jbtx is accompanied by changes in cGMP levels and in the transepithelial potential of Malpighian tubules (Stanisçuaski et al, 2009). Similar findings were reported by Martinelli et al (2014) for Jbtx. Urease and Jbtx target the *R. prolixus* immune system, inducing an eicosanoid-dependent aggregation of hemocytes and alterations in cell morphology that make the insect more susceptible to entomopathogenic bacteria (Defferrari et al, 2014; Fruttero et al, 2016).

Galvani et al (2015) used immunohistochemical techniques to demonstrate that Jbtx was distributed in the brain of *T. infestans*. Jbtx strongly inhibited the activity of nitric oxide synthase (NOS) in the central nervous system (CNS) and ganglion homogenates of these insects, leading to reduced levels of the neurotransmitter nitric oxide (NO); *in vitro* tests confirmed that Jbtx inhibited NOS activity. NO has an important role in neuronal function and may protect neurons against neurotoxicity (Calabrese et al, 2007; Sadekuzzaman et al, 2018). In contrast to the inhibition of NOS, Jbtx enhanced the *T. infestans* CNS activity of UDP-N-acetylglucosamine-pyrophosphorylase (UDP-GlcNAcP), an enzyme involved in glycosylation pathways and chitin synthesis, and also increased the activity of this enzyme in CNS homogenates of *D. peruvianus in vitro* in a concentration-dependent manner (Galvani et al, 2015).

Fruttero et al (2017) also investigated the effect of Jbtx on NOS and UDP-GlcNAcP activities in homogenates of CNS and salivary glands (SG) from *R. prolixus*, and examined the relationship between these alterations and gene expression. For NOS, incubation with Jbtx *in vitro* partially inhibited NOS activity while treatment *in vivo* (by feeding) inhibited this activity in the CNS, but not in SG. This finding implied a differential modulation of NOS in these organs, but this inhibition was not correlated with a decrease in the expression of NOS mRNA. Treatment with Jbtx *in vivo* and *in vitro* increased the activity of UDP-GlcNAcP in SG. However, in insects fed with Jbtx there was a decrease in the mRNA levels of UDP-GlcNAcP and chitin synthase, indicating a complex regulation exerted by this peptide on these enzymes. Moyetta et al (2017) reported that Jbtx enhanced the gene expression of UDP-GlcNAcP, NOS and chitin synthase *in vitro*, but no changes in gene expression or phosphorylation were seen *in vivo*. These authors also showed that Jbtx increased NO production in hemocyte aggregates (Moyetta et al, 2017).

dos Santos et al (2019) noted that Jbtx (8-32µg/gm body weight) causes bradycardia in semi-isolated heart preparations of *N. cinerea*, possibly by affecting octopaminergic pathways.

INTERACTION OF JBTX WITH LIPIDS AND MEMBRANES

Barros et al (2009) were the first to study the interaction of Jbtx with cell membranes, based on molecular modeling that demonstrated structural similarities between Jaburetox-2Ec and a β-hairpin peptide involved in the breakdown of the lipid bilayer. Martinelli et al (2014) developed three truncated versions of Jbtx that contained more than one domain of interaction with membrane lipids, a feature that could possibly contribute to the peptide´s toxicity (Barros et al, 2009; Martinelli et al, 2014; Kappaun et al, 2018). Structural analysis of JBU showed that an extensive region of Jbtx is exposed on the surface of JBU, which suggested that both the urease and its peptide have functional similarities in their insecticidal activity (Piovesan et al, 2014; Kappaun et al, 2018). Broll et al (2017) used Jbtx conjugated with fluorescein isothiocyanate (Jbtx-FITC) to examine the interactions between Jbtx and membrane lipids in *N. cinerea* and the yeast *Saccharomyces cerevisiae*; in addition, the structural behavior of the peptide was investigated by nuclear magnetic resonance spectroscopy and circular dichroism. Fluorescence microscopy revealed that Jbtx-FITC bound to *S. cerevisiae* as well as to the nerve cord of *N. cinerea*, thus confirming the affinity of Jbtx for cell membranes. The interaction of the peptide with fungal and insecticidal targets was thought to result in the formation of pores and/or alterations to tem cell membrane properties (Broll et al, 2017).

Micheletto et al (2016) investigated the interaction of JBU and Jbtx with platelet-like multilamellar liposomes (PML) using dynamic light scattering techniques and small-angle X-ray scattering, and also examined an effect on the hydrodynamic radius of vesicles. The results demonstrated that JBU interacted with PML by inserting its Jbtx domain into the liposome, causing a disturbance in the membrane. The insertion of Jbtx into the hydrophobic core of the membrane bilayer may: i) reduce the hydrodynamic radius of the vesicles; ii) alter the lamellar repetition distance; and iii) decrease the fluidity of the membrane, thereby affecting the organization of the internal bilayers (Micheletto et al, 2016; Kappaun et al, 2018).

ION CHANNELS AND JABURETOX

Several studies have examined the interaction of JBU, canatoxin and Jbtx with ion channels. Piovesan et al (2014) demonstrated the ability of JBU, Jbtx and versions of Jbtx to form ion channels in lipid bilayers. All of these channels displayed similar biophysical properties that consisted of two conducting states: ‘smaller channels’ with conductances of 7-18pS and ‘main channels’ with conductances of 32-79pS; all of these channels were highly selective for cations. These findings were confirmed by testing with planar lipid bilayers. The affinity of Jbtx for negatively charged membranes suggests that anionic lipids may constitute

possible receptors for the peptide. The fact that JBU and Jbtx share similar channel-forming properties strongly suggests that this peptide is located within the pore-forming domain(s) of the urease.

dos Santos et al (2019) reported that Jbtx markedly increased the amplitude of sodium currents in *X. laevis* oocytes over expressing BgNaV 1.1 channels from *Blattella germanica* (German cockroaches). The effects of the peptide on these channels is unclear since Jbtx did not facilitate twitch-tension responses in *N. cinerea* and *L. migratoria*. In contrast to these findings, Zanatta et al. (unpublished data) observed that Jbtx decreased the action potential amplitude by blocking sodium currents in isolated axons of the ventral nerve cord of American cockroaches (*P. americana*), without affecting potassium currents. These experiments were done using the single fiber double oil gap technique. Jbtx was also observed to cause permanent silencing of dorsal unpaired median (DUM) neurons, probably by blocking the sodium channels involved in the firing of these neurons.

The divergent effects of Jbtx on voltage-gated sodium channels noted above may be explained by the sensitivity of the techniques used. In the single fiber double oil gap technique, the axon is dissected from the ventral nerve cord of the animal and transferred to the recording chamber, where the experiments are done. The advantage of this technique is the ability to observe the effects of a toxin on the whole system while maintaining the wild-type characteristics of the channels (Stankiewicz et al, 2012). Although the single fiber double oil gap technique is not as sensitive as voltage-clamp recordings in oocytes (Rubaiy, 2017), the retention of wild-type characteristics of the channels when working with whole tissues excludes the possibility of some ancillary channel subunits being omitted, such as may occur during coexpression in oocytes (Pongs and Schwarz, 2010). Further biochemical, electrophysiological and molecular experiments are required to elucidate the action of Jbtx on ion channels.

CONCLUSIONS

The studies discussed above indicate that Jbtx induces a wide variety of neurological manifestations in insects that include alterations in antennal and grooming activity, diuresis, bradycardia and neuromuscular blockade. These changes may be mediated by: i) direct modulation of voltage-gated sodium channels in the ventral nerve cord to influence octopaminergic neurotransmission; and ii) alterations in the expression and/or activity of key enzymes such as AChE, NOS and UDP-GlcNAcP in the central and peripheral nervous systems. Octopamine is the major neurotransmitter of efferent nerves arising from metha-, meso- and prothoracic ganglia that modulate GABA and GLU at insect neuromuscular junctions; octopamine can also

activate the subesophageal ganglion to interfere with leg and antennal grooming (Figure 2). Some of these effects are shared with most of the *C. ensiformis* ureases, while others are exclusive to Jbtx. Figure 3 summarizes the range of effects caused by Jbtx in insects.

The various alterations caused by Jbtx in insects suggests the potential use of this peptide as a natural pesticide to protect plants against insect pests and fungi. Indeed, preliminary results for soybean, corn and sugar cane plants that express Jbtx have indicated an improvement in plant resistance to insect pests (Carlini and Ligabue-Braun, 2016). An assessment of the potential risks associated with biotechnological applications of Jbtx has recently been done based on a tiered weight-of-evidence approach, following the recommendations of the International Life Sciences Institute. The results revealed the selectivity of the peptide against insects and fungi, its susceptibility to digestion and no relevant similarity to any known toxic, antinutritional or allergenic proteins (Sá et al, 2020). These findings agreed with the previous demonstration that Jbtx was harmless to mice and rats in high-dose acute toxicity tests (Mulinari et al, 2007). Additional investigations are needed to elucidate the precise mechanism of action of this peptide and to fully establish its safety in biotechnological applications.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGMENTS

YCB, MER, APZ and BTB were supported by scholarships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil; Finance Code 001). This work was supported by a grant from CAPES (Edital 063/2010 Toxinologia).

REFERENCES

Barros PR, Stassen H, Freitas MS, Carlini CR, Nascimento MAC and Follmer C. 2009. Membrane-disruptive properties of the bioinsecticide Jaburetox-2Ec: implications to the mechanism of the action of insecticidal peptides derived from ureases. Biochim Biophys Acta – Proteins Proteomics, 1794, 1848-1854.

Broll V, Martinelli AHS, Lopes FC, et al. 2017. Structural analysis of the interaction between Jaburetox, an intrinsically disordered protein, and membrane models. Colloids Surf. B Biointerfaces, 159, 849-860.

295 Calabrese V, Mancuso C, Calvani M, Rizzarelli E, Butterfield DA and Giuffrida Stella AM. 2007. Nitric oxide in
296 the central nervous system: neuroprotection versus neurotoxicity. Nat Rev Neurosci, 8, 766-775.

297 Callahan BP, Yuan Y and Wolfenden R. 2005. The burden borne by urease. J Am Chem Soc, 127, 10828-
298 10829.

299 Carlini CR and Guimarães JA. 1981. Isolation and characterization of a toxic protein from *Canavalia*
300 *ensiformis* (jack bean) seeds, distinct from concanavalin A. Toxicon, 19, 667-675.

301 Carlini CR and Ligabue-Braun R. 2016. Ureases as multifunctional toxic proteins: a review. Toxicon, 110, 90-
302 109.

303 Carlini CR, Oliveira AEA, Azambuja P, Xavier-Filho J and Wells MA. 1997. Biological effects of canatoxin in
304 different insect models: evidence for a proteolytic activation of the toxin by insect cathepsin-like enzymes. J
305 Econ Entomol, 90, 340-348.

306 Carter EL, Flugga N, Boer JL, Mulrooney SB and Hausinger RP. 2009. Interplay of metal ions and urease.
307 Metallomics, 1, 207-221.

308 Carter EL, Tronrud DE, Taber SR, Karplus PA and Hausinger RP. 2011. Iron-containing urease in a pathogenic
309 bacterium. Proc Natl Acad Sci USA, 108, 13095-13099.

310 Costa LG, Giordano G, Guizzetti M and Vitalone A. 2008. Neurotoxicity of pesticides: a brief review. Front
311 Biosci, 13, 1240-1249.

312 Defferrari MS, da Silva R, Orchard I and Carlini CR. 2014. Jack bean (*Canavalia ensiformis*) urease induces
313 eicosanoid-modulated hemocyte aggregation in the Chagas' disease vector *Rhodnius prolixus*. Toxicon, 82,
314 18-25.

315 Dixon NE, Gazzola C, Blakeley RL and Zerner B. 1975. Jack bean urease (EC 3.5.1.5). A metalloenzyme. A
316 simple biological role for nickel? J Am Chem Soc, 97, 4131-4133.

317 dos Santos DS, Zanatta AP, Martinelli AHS, et al. 2019. Jaburetox, a natural insecticide derived from jack
318 bean urease, activates voltage-gated sodium channels to modulate insect behavior. Pestic Biochem Physiol,
319 153, 67-76.

320 Follmer C, Carlini CR, Yoneama ML and Dias JF. 2002. PIXE analysis of urease isoenzymes isolated from
321 *Canavalia ensiformis* (jack bean) seeds. Nucl Instrum Methods Phys Res B, 189, 482-486.

322 Franco R, Li S, Rodriguez-Rocha H, Burns M and Panayiotidis MI. 2010. Molecular mechanisms of pesticide-
323 induced neurotoxicity: relevance to Parkinson's disease. Chem Biol Interact, 188, 289-300.

324 Fruttero LL, Moyetta NR, Uberti AF, et al. 2016. Humoral and cellular immune responses induced by the
325 urease-derived peptide Jaburetox in the model organism *Rhodnius prolixus*. Parasit Vectors, 9, 412.

326 Fruttero LL, Moyetta NR, Krug MS, et al. 2017. Jaburetox affects gene expression and enzyme activities in

Rhodnius prolixus, a Chagas' disease vector. Acta Trop, 168, 54-63.

Galvani GL, Fruttero LL and Coronel MF. 2015. Effect of the urease-derived peptide Jaburetox on the central nervous system of *Triatoma infestans* (Insecta: Heteroptera). Biochim Biophys Acta - Gen Subj, 1850, 255-262.

Islam A and Malik MF. 2018. Toxicity of pesticides on CNS. J Toxicol Anal 1, 1-7.

Kappaun K, Piovesan AR, Carlini CR and Ligabue-Braun R. 2018. Ureases: historical aspects, catalytic, and non-catalytic properties – A review. J Adv Res, 13, 3-17.

Krajewska B. 2009. Ureases I. Functional, catalytic and kinetic properties: a review. J Mol Catal B Enzym, 59, 9-21.

Ligabue-Braun R, Andreis FC, Verli H and Carlini CR. 2013. 3-to-1: unraveling structural transitions in ureases. Naturwissenschaften, 100, 459-467.

Martinelli AHS, Kappaun K, Ligabue-Braun R, et al. 2014. Structure-function studies on jaburetox, a recombinant insecticidal peptide derived from jack bean (*Canavalia ensiformis*) urease. Biochim Biophys Acta - Gen Subj, 1840, 935-944.

Micheletto YMS, Moro CF, Lopes FC, et al. 2016. Interaction of jack bean (*Canavalia ensiformis*) urease and a derived peptide with lipid vesicles. Colloids Surf B Biointerfaces, 145, 576-585.

Moyetta NR, Broll V, Perin APA, et al. 2017. Jaburetox-induced toxic effects on the hemocytes of *Rhodnius prolixus* (Hemiptera: Reduviidae). Comp Biochem Physiol C Toxicol Pharmacol 200, 17-26.

Mulinari F, Becker-Ritt AB, Demartini DR, et al. 2011. Characterization of JBURE-IIb isoform of *Canavalia ensiformis* (L.) DC urease. Biochim Biophys Acta - Proteins Proteomics, 1814, 1758-1768.

Mulinari F, Stanisçuaski F, Bertholdo-Vargas LR, et al. 2007. Jaburetox-2Ec: an insecticidal peptide derived from an isoform of urease from the plant *Canavalia ensiformis*. Peptides, 28, 2042-2050.

Ngai M and McDowell MA. 2017. The search for novel insecticide targets in the post-genomics era, with a specific focus on G-protein coupled receptors. Mem Inst Oswaldo Cruz, 112, 1-7.

Piovesan AR, Martinelli AHS, Ligabue-Braun R, Schwartz JL and Carlini CR. 2014. *Canavalia ensiformis* urease, jaburetox and derived peptides form ion channels in planar lipid bilayers. Arch Biochem Biophys, 547, 6-17.

Pongs O and Schwarz JR. 2010. Ancillary subunits associated with voltage-dependent K^+ channels. Physiol Rev 90, 755-796.

Postal M, Martinelli AHS, Becker-Ritt AB, et al. 2012. Antifungal properties of *Canavalia ensiformis* urease and derived peptides. Peptides, 38, 22-32.

358 Rubaiy HN. 2017. A short guide to electrophysiology and ion channels. J Pharm Pharm Sci, 20, 48-67.

359 Sá CA, Vieira LR, Pereira Almeida Filho LC, et al. 2020. Risk assessment of the antifungal and insecticidal
360 peptide jaburetox and its parental protein the jack bean (*Canavalia ensiformis*) urease. Food Chem Toxicol,
361 136, 110977.

362 Sadekuzzaman M, Stanley D and Kim Y. 2018. Nitric oxide mediates insect cellular immunity via
363 phospholipase A_2 activation. J. Innate Immun. 10, 70-81.

364 Sirko A and Brodzik R. 2000. Plant ureases: roles and regulation. Acta Biochim Pol 47, 1189-1195.

365 Stanisçuaski F, Te Brugge V, Carlini CR and Orchard I. 2009. In vitro effect of *Canavalia ensiformis* urease
366 and the derived peptide Jaburetox-2Ec on *Rhodnius prolixus* Malpighian tubules. J Insect Physiol 55, 255-
367 263.

368 Stankiewicz M, Dąbrowski M and de Lima ME. 2012. Nervous system of *Periplaneta americana* cockroach as
369 a model in toxinological studies: a short historical and actual view. J Toxicol 2012, 1-11.

370 Sumner JB. 1926. The isolation and crystallization of the enzyme urease. J Biol Chem 69, 435-441.

371 Tomazetto G, Mulinari F, Stanisçuaski F, Settembrini B, Carlini CR and Ayub MAZ. 2007. Expression kinetics
372 and plasmid stability of recombinant *E. coli* encoding urease-derived peptide with bioinsecticide activity.
373 Enzyme Microb Technol 41, 821-827.

374 Zambelli B, Musiani F, Benini S and Ciurli S. 2011. Chemistry of Ni^{2+} in urease: sensing, trafficking, and
375 catalysis. Acc Chem Res 44, 520-530.

Figures

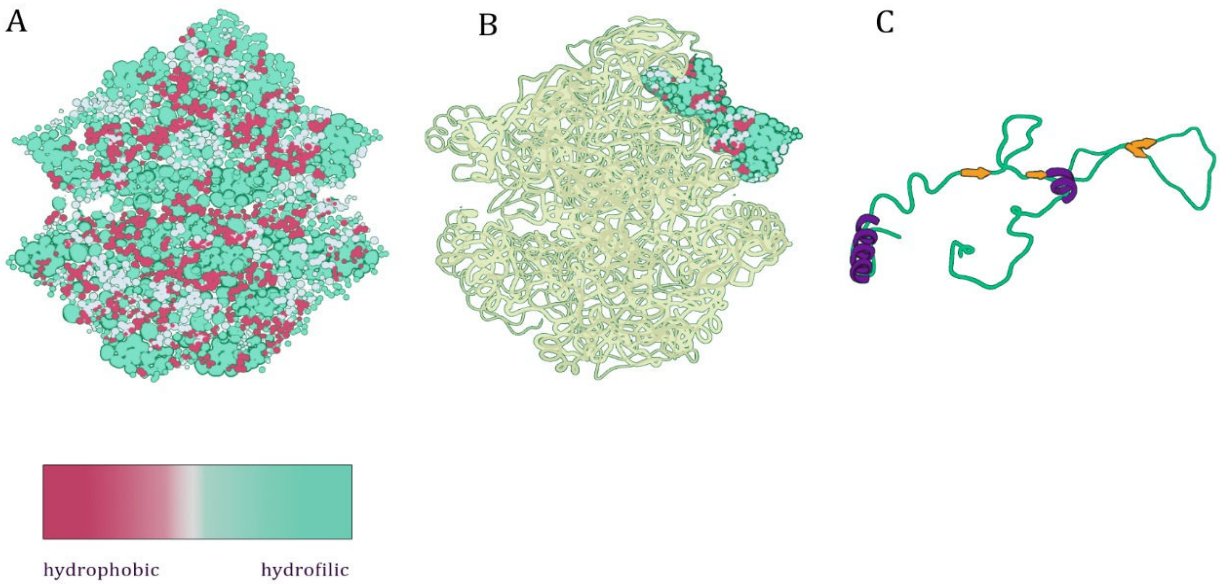

Figure 1. Illustration of the general structural organization and surface properties of the jack bean urease (JBU) hexamer, based on Micheletto et al (2016). **A.** Hydrophobicity of JBU, colored according to the Kyte-Doolittle scale (Red, hydrophobic; light blue, hydrophilic); "Side" views of the oligomer are relative rather than absolute representations. **B.** The location of the jaburetox sequence in one of the monomers shown in the same orientation as the "side" view. **C.** The crystal-derived structure of jaburetox obtained from intact JBU.

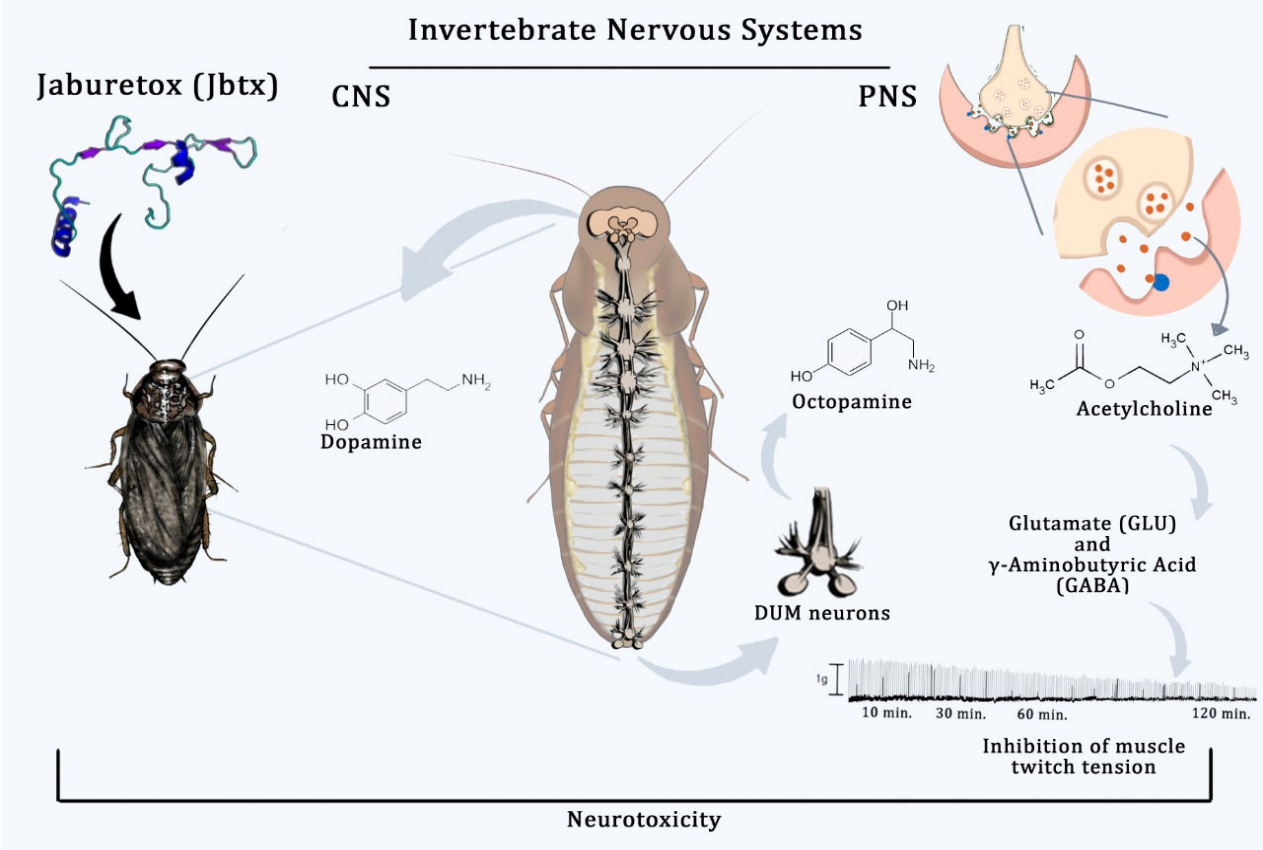

Figure 2. Neuropharmacology of the insect nervous system. Octopamine is a major neurotransmitter in insects, the release of which is modulated by the activation of voltage-gated sodium channels in dorsal unpaired median (DUM) neurons present in the ventral nerve cord. As a neuromodulator, octopamine stimulates efferent nerves of thoracic ganglia to cause the release of g-amino-butyric acid (GABA) and glutamate (GLU) at neuromuscular junctions, potentially via cholinergic nerves. Octopamine directly stimulates leg grooming activity and indirectly stimulates antennal grooming via dopamine release. CNS – central nervous system, PNS – peripheral nervous system.

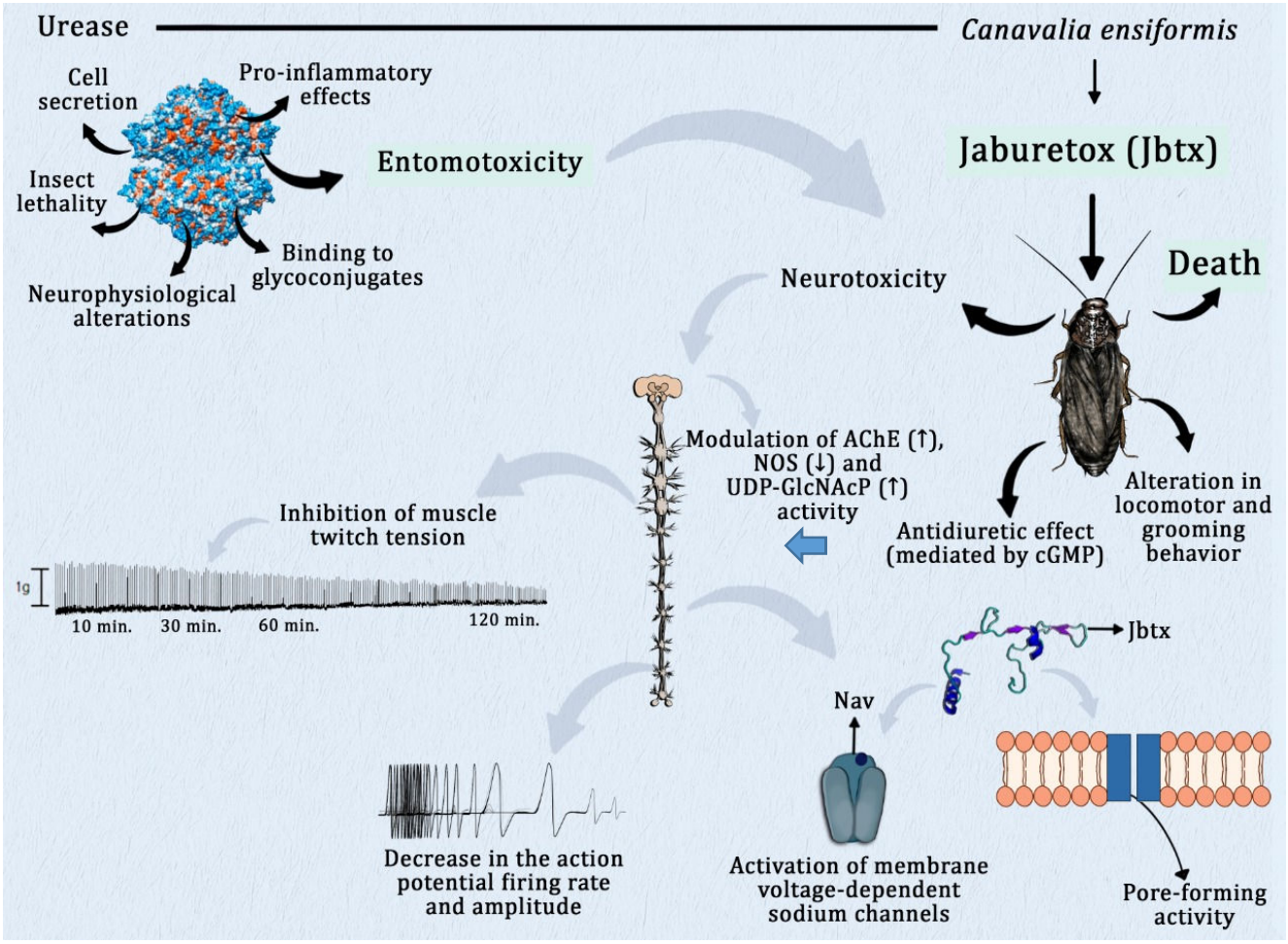

Figure 3. A summary of the main neurotoxic effects of jaburetox (Jbtx) in insects. These effects include the modulation of locomotor and grooming behavior, an antidiuretic effect, neuromuscular blockade, a decrease in the frequency and amplitude of neuronal action potentials, modulation of voltage-gated sodium channels (Nav), pore formation and an increase or decrease in enzyme activity. Note that some of these actions are shared with jack bean urease (JBU) (top left) while others differ from JBU (not shown here).