



Dopaminergic metabolism is affected by intracerebral injection of Tb II-I isolated from *Tityus bahiensis* scorpion venom

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ARTICLE INFO

Handling Editor: Dr. Denise Tambourgi

Keywords:

Neurotoxin
Tityus bahiensis
Hippocampus
Neurotransmitters
Dopaminergic system

ABSTRACT

Tb II-I isolated from *Tityus bahiensis* venom causes epileptic discharges when injected into the hippocampus of rats. The involvement of neurotransmitters in this activity was investigated. Our results demonstrated that Tb II-I increases the concentrations of dopamine metabolite but does not alter other neurotransmitters. Thus, dopaminergic system seems to be partially responsible for the convulsive process. Specific action on particular neurotransmitter can make this toxin a useful tool to better understand the functioning of the system.

Scorpions are the main responsible for human accidents with venomous animals in Brazil, being *Tityus serrulatus* and *T. bahiensis* (Tb) the most relevant species.

The composition and characterization of the venoms of some scorpions have been studied for a long time and consist of a complex mixture of small organic molecules, proteins and peptides, the last ones, also known as neurotoxins (Bertazzi et al., 2003; Cologna et al., 2009), causing disturbances in the normal physiology of tissues such as muscles and nerves through their interaction with ion channels (Tian et al., 2008).

Isolated toxins from Tb venom have not yet been fully characterized and their effects on the central nervous system (CNS) are poorly understood. Some studies have demonstrated that peripheral Tb crude venom injection causes electroencephalographic and behavioral alterations (Nencioni et al., 2009); fractions of this venom cause similar alterations and neuronal loss (Lourenço et al., 2002); and some isolated toxins, when directly injected into the hippocampus of rats, induce wet dog shake (WDS), myoclonus, epileptic discharges (Ossanai et al., 2012) and increase in the intracerebral level of glutamate (Beraldo Neto et al., 2020).

In a previous study we have demonstrated that the fraction Tb II-I, isolated from Tb venom and composed by two toxins, Tb4 and Tb2-II (Beraldo Neto et al., 2018), causes convulsive behavior, neuronal loss and alteration in the levels of cytokines in the CNS of rats when

intrahippocampally injected (Beraldo Neto et al., 2018). Now, we aimed to complement this study (Beraldo-Neto et al., 2018) assessing the intracerebral levels of some neurotransmitters after the intrahippocampal injection of this fraction, in order to understand if they are responsible for the alterations described above.

The lyophilized venom was provided by the Strategic Nucleus of Venoms and Antivenoms, Butantan Institute São Paulo – Brazil; and the fractionation was obtained according to previously described by our group (Beraldo Neto et al., 2018).

Seven male Wistar rats (260–280 g) provided by the Central Animal Facility of the Butantan Institute and maintained under controlled conditions (light with cycles of 12 h light/dark and temperature of 22 ± 2 °C, water and food ad libitum) were used. All the procedures were previously approved by the Institutional Ethics Committee for Experimental Animals (No. 1389/15). The animals were intraperitoneally anesthetized with 10% Ketamine Hydrochloride (Syntec, São Paulo, Brazil) and 2% Xilazine Hydrochloride (Syntec, São Paulo, Brazil). A CMA/11 guide cannula with a replaceable inner guide (CMA Microdialysis, Stockholm, Sweden) was stereotaxically implanted in the left hippocampus. The coordinates (AP –5.3, L –4.0, V –2.0.) were established according to the Stereotaxic Atlas of Paxinos and Watson (1998) and the surgical method was performed according to previous works (Beraldo Neto et al., 2020; Nencioni et al., 2009; Ossanai et al., 2012).

After recovery of the animals, guide cannula obturator was replaced

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<https://doi.org/10.1016/j.toxcx.2022.100126>

Received 5 November 2021; Received in revised form 22 April 2022; Accepted 17 May 2022

Available online 27 May 2022

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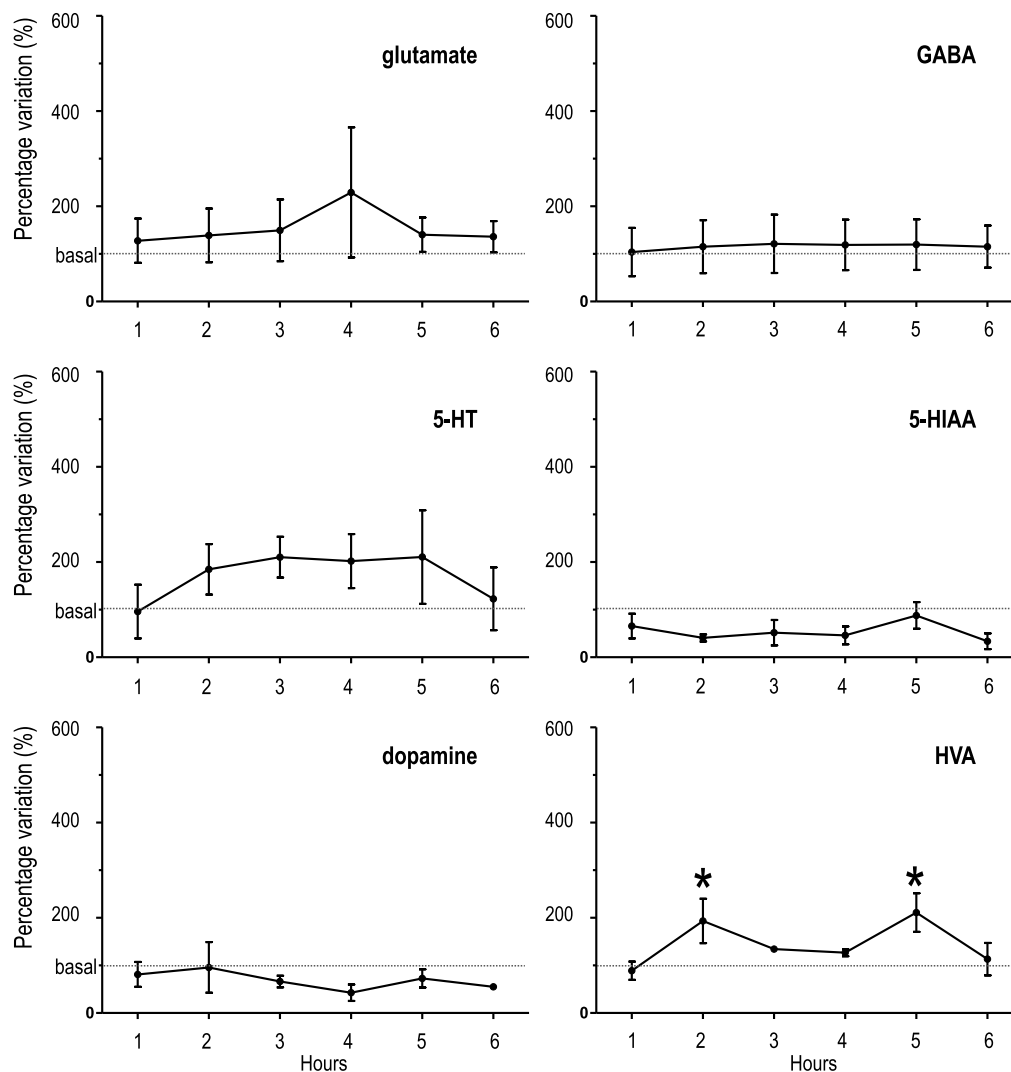


Fig. 1. Extracellular levels of glutamate, GABA, 5-HT, DA, and metabolites as determined by microdialysis in the CA1 area of the hippocampus in conscious rats ($n = 7$) before and after injection of $2 \mu\text{g}/2 \mu\text{L}$ of Tb II-I in the same area. Line corresponds to the average of three basal collections which was used to establish baseline values. Data are represented as the means + SD of the mean. ANOVA for repeated measures followed by Tukey's test. * $p < 0.05$.

by a 2-mm CMA 11 microdialysis probe (CMA Microdialysis, Stockholm, Sweden), and continuous perfusion was started with Ringer's solution (rate $2.13 \mu\text{L}/\text{min}$; CMA 100 Microdialysis pump). Dialysates were collected every 60 min from the freely moving animals. The microdialysis experiment was performed in three steps: (1) three basal collections, the average of which was used to establish baseline values, (2) intracerebral injection of Tb II-I ($2 \mu\text{g}/2 \mu\text{L}$), and (3) six more sample collections, making a total of 9 per animal. The samples were immediately frozen and maintained in $-80 \text{ }^\circ\text{C}$ until analysis. All dialysate samples were split for analysis of GABA and glutamate, and monoamines dopamine (DA) and 5-hydroxytryptamine (5-HT) and their metabolites homovanilic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA).

For monoamines analysis a solution containing 1 M perchloric acid, 0.2% EDTA and 0.2% sodium metabisulfite was added to the dialysate (1:9, v:v). Samples were analyzed by RP-HPLC with ionic annealing (Shimadzu Model 20 A, Kyoto Japan) coupled to an electrochemical detector (Antek-Decade, Zoeterwoude, The Netherlands) with a ODS C18 column ($150 \times 4.6 \text{ mm}$ Shimpak, Kyoto Japan) and a line filter was used. For amino acids analysis dialysates did not receive any pre-treatment. The samples were derivatized employing 70% ethanol, 10% triethylamine and 10% phenylisothiocyanate solution. $50 \mu\text{L}$ of the samples were injected in UFLC (Shimadzu, Kyoto Japan) coupled with a

reverse phase C18 column with a variable UV detector using a 254 nm wavelength for sample reading. The neurotransmitters were identified according to their retention time, comparing to a known concentration standard.

Analysis of glutamate and GABA levels demonstrated that there were no statistically significant differences between the baseline samples and those collected after the injection of the fraction (Fig. 1). The other neurotransmitters or metabolites were not significantly altered either (Fig. 1) except HVA, that increased compared to baseline (the average of the three first collections), being statistically significant at the fifth and eighth hour (Fig. 1).

Venoms generally act on the peripheral nervous system in order to paralyze preys or predators. But the action in the CNS cannot be ruled out, as demonstrated in this study. Although the CNS is probably not the main focus of the animal, this type of toxins can be a useful pharmacological tool for study this system.

As previously demonstrated, the fraction Tb II-I causes epileptic-like behavior, electrographic alterations such as grouped spikes and strong discharges, alters the level of some cytokines in the hippocampus, and additionally decreases the number of pyramidal cells in the CA1, CA3 and CA4 hippocampal areas (Beraldo Neto et al., 2018). These effects are commonly associated with increase in the level of the glutamate, the

main neurotransmitter associated with continued depolarization caused by scorpionic neuropeptides (Beraldo Neto et al., 2020; Massensini et al., 1998; Nencioni et al., 2003, 2009). However, in the present study we demonstrated an opposite effect, with no changes in the intra-hippocampal level of glutamate and GABA. Instead, it was observed an increase in the level of extracellular HVA, suggesting an alteration in dopaminergic metabolism (Wiesel et al., 1973) since drugs that cause dopamine release, increase the concentration of HVA often without changing the dopamine concentration (Freitas et al., 2004; Kopin et al., 1988; Starr, 1996). Inflammatory cytokines affect the dopamine metabolism (Felger and Miller, 2012; Miller et al., 2013) and the previously observed changes in IL-6 and TNF- α level (Beraldo Neto et al., 2018), could be partially responsible for this result.

Our results are in accordance with previous works with scorpion venoms, in which it was observed an increase in HVA but not in DA levels, in striatum and hypothalamus after intravenous injection of *T. serrulatus* venom (Dorce and Sandoval, 1994), or an increase in intra-hippocampal levels of HVA after peripheral injection of *T. serrulatus* and *T. bahiensis* venoms (Nencioni et al., 2009). It was also demonstrated the release of DA from cortical slices of rat brain induced by tityustoxin and TITX γ toxins, isolated from *T. serrulatus* venom (Fernandes et al., 2004a; 2004b).

Monoaminergic systems are the target for many venomous animals which act inducing or blocking the release of monoamine from cells, blocking the reuptake, affecting synthesis, acting as agonists and antagonists at monoaminergic receptors, and changing sensitivity of receptors (Weisel-Eichler and Libersat, 2004). Monoaminergic systems act as neuromodulators in the CNS (Strac et al., 2016) and DA has a prominent role in limbic seizures since several experimental models point DA as one of the factors increased (Meurs et al., 2008). Particularly, activation of D1-type receptors reduces the threshold and aggravates the seriousness of seizures in animal models of acquired epilepsy (DeNinno et al., 1991; Gangarossa et al., 2014), which induce generalized convulsions. Activation of D1-like receptors increases cAMP levels and protein kinase A (PKA) activity via the stimulation of adenylyl cyclase, and consequently the phosphoprotein DARPP-32 is activated (Bozzi and Borelli, 2013). This protein activates a series of signaling cascades that are important in regulating neuronal excitability (Bozzi et al., 2011).

Our results lead us to believe that the pro-convulsive effects of Tb II-I previously described (Beraldo Neto et al., 2018) is at least in part the consequence of an overstimulation of the dopaminergic system.

This result is particularly important because highlights the specificity of action of Tb II-I and the discovery of selective toxins that affect the dopaminergic system can be useful and help to identify possible therapeutic targets, since several neuropsychiatric symptoms are mediated by changes in dopaminergic function (Miller et al., 2013).

Our findings can be resumed in (1) an increase in the release HVA; and (2) in the stagnation in the levels of glutamate and GABA, which are important neurotransmitters related to envenoming. The observed result generates complex discussion since there are few studies linking toxins and the central nervous system, particularly with regard to neurotransmitters and cytokines.

Author's contribution

Emídio Beraldo Neto. I declare that I participated in the study by planning and developing the purification of the venom and neurotransmitters quantification, analyzing the results and preparing the manuscript for publication and that I have seen and approved the final version. I declare that there are no conflicts of interest.

Ivo Lebrun. I declare that I participated in the study by planning and developing the neurotransmitters quantification and analyzing the results and that I have seen and approved the final version. I declare that there are no conflicts of interest.

Ana Leonor Abrahão Nencioni. I declare that I participated in the

study by planning the experiments, analyzing the results and preparing the manuscript for publication, and that I have seen and approved the final version. I declare that there are no conflicts of interest.

Funding

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível superior (CAPES)/Toxinology program (AUX-PE-Toxinologia-1207/2011).

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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