



HAL
open science

Cyriotoxin-1a, the first toxin with antinociceptive properties purified from *Cyriopagopus schioedtei* spider venom: from identification to structural and functional characterization

Tânia C. Gonçalves, Evelyne Benoit, Michael Kurz, Laetitia Lucarain, Sophie Fouconnier, Stéphanie Combemale, Lucie Jaquillard, Brigitte Schombert, Rachid Boukaiba, Gerhard Hessler, et al.

► **To cite this version:**

Tânia C. Gonçalves, Evelyne Benoit, Michael Kurz, Laetitia Lucarain, Sophie Fouconnier, et al.. Cyriotoxin-1a, the first toxin with antinociceptive properties purified from *Cyriopagopus schioedtei* spider venom: from identification to structural and functional characterization. Gordon Research Conference - Venom Evolution, Function and Biomedical Applications, Aug 2018, West Dover VT, United States. . cea-04466926

HAL Id: cea-04466926

<https://cea.hal.science/cea-04466926>

Submitted on 19 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

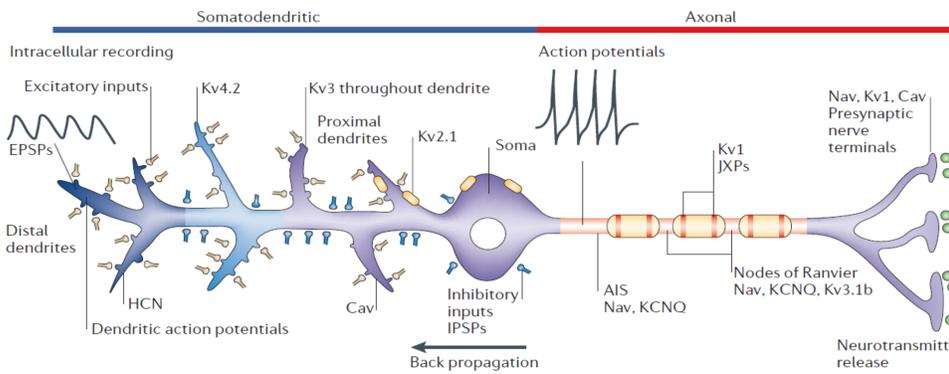
Cyriotoxin-1a, the first toxin with antinociceptive properties purified from *Cyriopagopus schioedtei* spider venom: from identification to structural and functional characterization

T. Gonçalves^{a,c}, E. Benoit^{c,d}, M. Kurtz^b, L. Lucarain^a, S. Fouconnier^a, S Combemale^d, L. Jaquillard^d, B. Schombert^a, R. Boukaiba^a, G. Hessler^d, A. Bohme^a, L. Bialy^e, S. Hourcade^e, R. Bérout^f, M. De Waard^f, D. Servent^{c,d}, M. Partiseti^a & JM. Chambard^a

Sanofi Aventis R & D, Integrated Drug Discovery – High Content Biology, Vitry-sur-Seine, France ^a / Synthetic Molecular Design, Frankfurt, Germany ^b
 Service d'Ingénierie Moléculaire des Protéines (SIMOPRO), CEA, Université Paris-Saclay, Gif sur Yvette, France ^c
 Institut des Neurosciences Paris-Saclay (Neuro-PSI), UMR CNRS/Université Paris-Sud 9197, Université Paris-Saclay, Gif sur Yvette, France ^d
 Sanofi R & D, Neuroscience Therapeutic Area, Neurodegeneration Research, F-91385, Chilly-Mazarin, France ^e
 Smarttox Biotechnology, 6 rue des Platanes, F-38120 Saint-Egrève, France ^f



Na_v1.7 : an antinociceptive target genetically validated

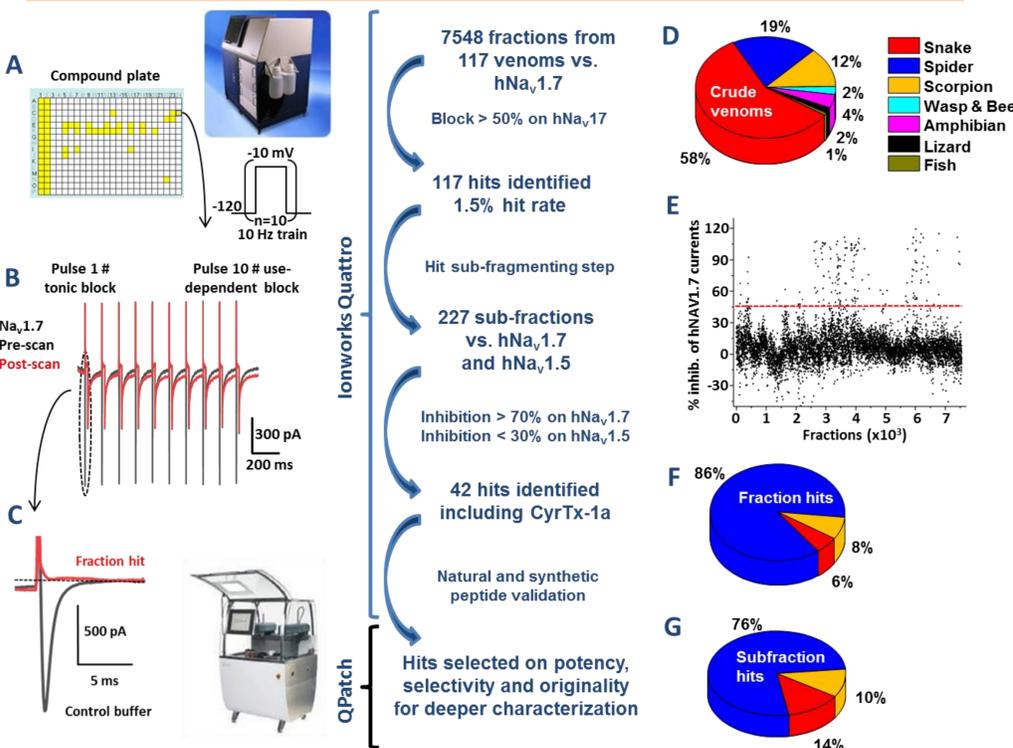


Na_v1.7 is mainly expressed in dorsal root ganglia (DRG) neurons from peripheral to central nerve terminals (PNS). The secondary expression takes place in olfactory sensory nerves (PNS), in the pituitary gland and the subfornical organ as well as several hypothalamic nuclei (CNS) (cartoon from Lai *et al.* 2006)

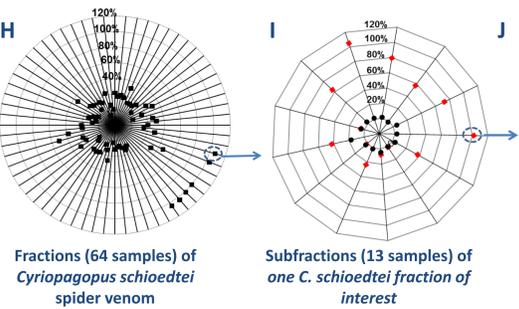
Human Na_v1.7 genetic alterations are linked to pain :

- Na_v1.7 loss of function is the root of congenital insensitivity to pain (CIP) or hereditary sensory and autonomic neuropathy (HSAN) type IID.
- Na_v1.7 gain of function can cause hypersensitivity to pain , primary erythromelalgia (PE), paroxysmal extreme pain disorder (PEPD), as well as small fiber neuropathy.

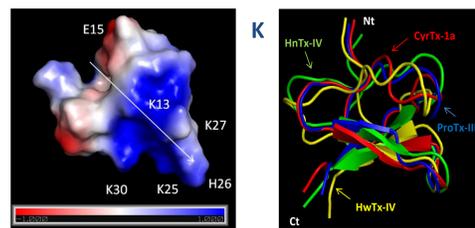
In-vitro venom screening workflow



(A) Collection of venoms was prepared into fractions individually added to 384-well plates (columns 1 and 2 are TTX full block and columns 3 and 4 are maximal current obtained in extracellular buffer) for testing vs. hNa_v1.7 on the Ionworks Quattro (IWQ, Molecular Devices). The train protocol (described as an insert) was applied before and after 10-min fraction incubation. Traces of pre-and post-scan are shown in full (B). Tonic block obtained on hNa_v1.7 has been enlarged (C). Source of the screened crude venom is shown in (D). Percentage of inhibition for all tested fractions was plotted. As shown in (E), a threshold was set at 50% block (red line). From the venom library tested, only 8% hits versus hNa_v1.7 came from scorpion venom, 6% from snake while the larger majority (86%) was spider fractions (F). (G) Hits were sub-fractionated and tested again versus hNa_v1.7 and hNa_v1.5 to identify most promising hits to be further characterized using whole-cell patch-clamp aPC (QPatch, Sophion).

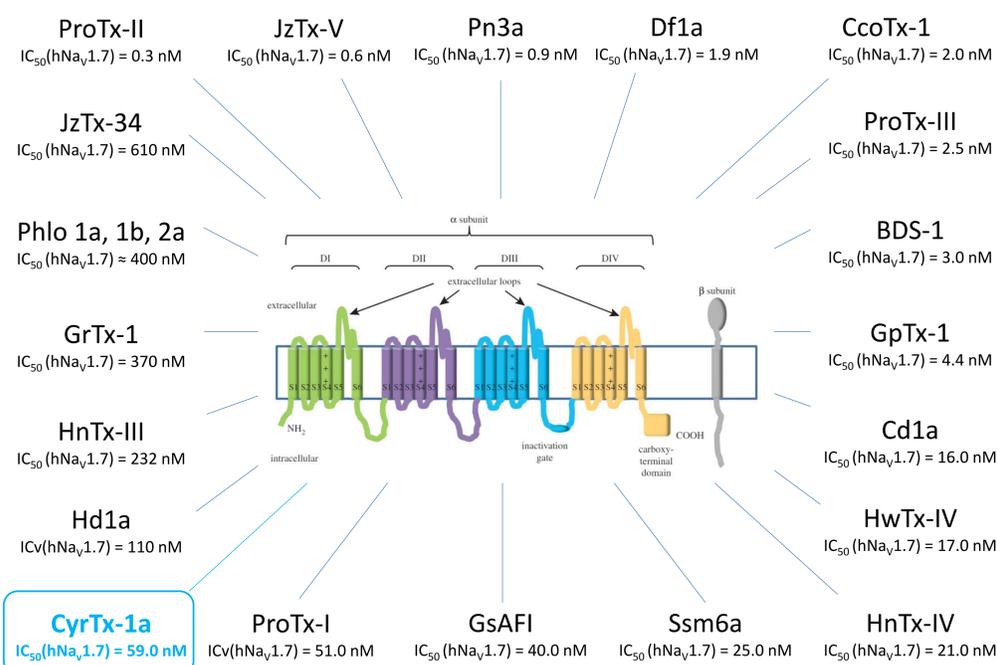


Flowchart for hNa_v1.7 hit peptide identification. (H) Percentage of inhibition of peak hNa_v1.7 elicited current by application of 0.05 µg of each of the 64 fractions obtained from *C. schioedtei* spider venom. (I) Each fraction was then separated again to isolate one peptide per well. Sub-fractions were tested again in our IWQ assay. At this stage, hNa_v1.7 (red squares) and hNa_v1.5 (black circles) inhibition was investigated.



Representation of CyrTx-1a identified by PyMOL. (J) Electrostatic charged surface representation of CyrTx-1a. The molecule is rendered as a surface colored according to the electrostatic potential. The entire structure has a clear dipole potential with E1 and E15 forming a negative zone while K3, K7, K13, K25, H26, K27 and K30 form a positive zone. (K) Superposition of backbone peptide folding of CyrTx-1a and three other toxins of the NaSpTx family I previously described to possess analgesic effects (PDB entries of HnTx-IV: 1NIY, ProTx-III: 2MXM and HwTx-IV: 1MB6).

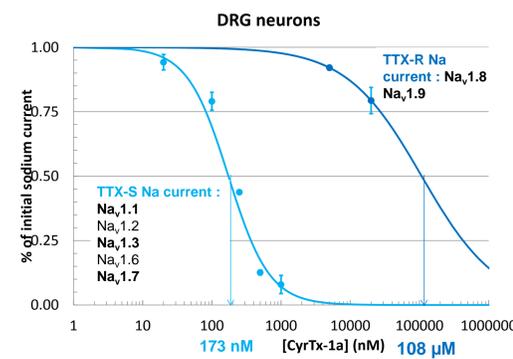
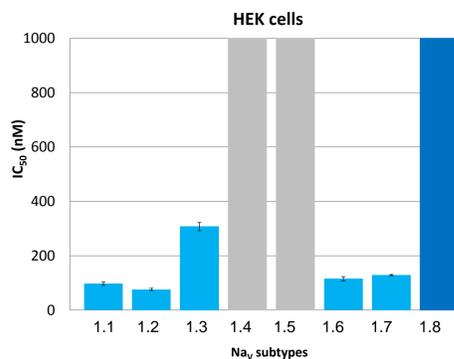
Inhibitory toxins : potential painkillers targeting Na_v1.7



Spider venom provides an amount of highly specific inhibitory cystine knots (ICK)-patterned toxins inhibiting Na_v1.7 with a natural selectivity against Na_v1.5 (> 2 logs). The BDS-1 and the Ssm6a are provided from sea anemone and Chinese red-headed centipede venoms, respectively.

In-vitro selectivity characterization of CyrTx-1a

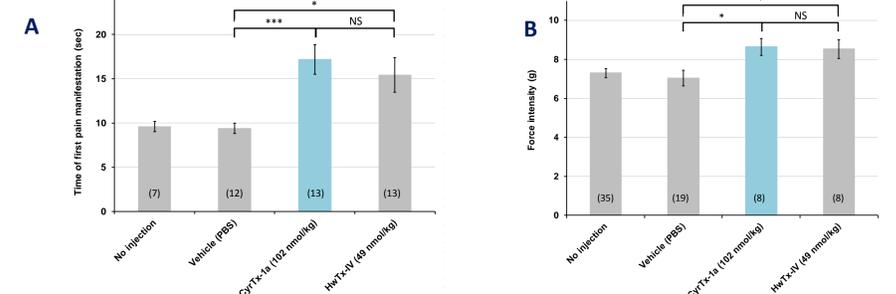
The peptide exhibits nanomolar range affinity for Na_v1.7, Na_v1.1-1.3 and Na_v1.6 subtypes and micromolar range affinity for Na_v1.5 and Na_v1.4 subtypes in HEK293-Na_v engineered cell lines.



In DRG neurons isolated from adult mice, the CyrTx-1a peptide preferentially inhibited with very high affinity tetrodotoxin (TTX)-sensitive Na_v currents, mainly represented by the Na_v1.7 subtype.

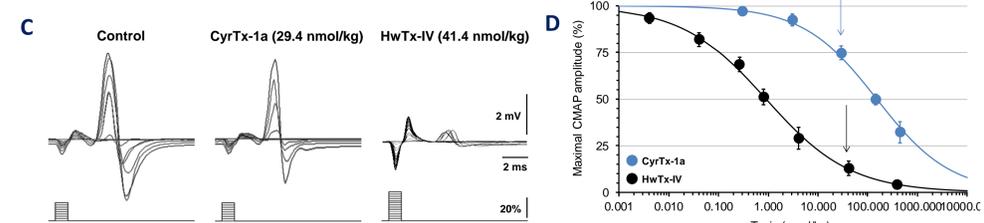
In-vivo mouse studies using CyrTx-1a

Evaluation of painkiller effects



The hot plate (A, thermic pain) and Von Frey (B, mechanic pain) assays are acute pain tests. The hot plate and von Frey pain assays showed that mice injected with 367 µg/kg of CyrTx-1a or with 200 µg/kg of HwTx-IV were more resistant to pain than animals injected with the vehicle (PBS).

Evaluation of muscle side-effects



The compound muscle action potential (C) assessment of both toxin reveals a narrow therapeutic window (D) with lethality occurring after the painkiller dose probably due to muscles (including diaphragm) paralysis.

Take home messages

- As Na_v1.7 is involved in a multitude of genetic pain disorders, it seems obvious that this sodium channel subtype might be an important antinociceptive target.
- Toxins from spider venoms are valuable tools with characteristics of high affinity against Na_v1.7 and high selectivity against Na_v1.5. The studies of all these toxin sequences will help us better understand which amino acids are crucial for the binding on Na_v1.7 and which could increase selectivity against closely-related ion channels.
- CyrTx-1a is a promising toxin targeting Na_v1.7 with a nanomolar potency (selective vs. TTX-R NaV channels) and exhibiting antinociceptive *in-vivo* effects. However some structure-activity relationship studies are required to exclude the muscle contraction side-effects probably caused by the high affinity of CyrTx-1a for Na_v1.6 expressed in nodes of Ranvier of alpha motoneurons.