

Microtransplantation of tick transmembrane proteins as a strategy to screen new compounds and to study transmembrane proteins from arthropod vectors of diseases

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SCIENCE & IMPACT

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ΛGENCE

INNOVATION

DÉFENSE Microtransplantation of tick transmembrane proteins as a strategy to screen new compounds and to study transmembrane proteins from arthropod vectors of diseases



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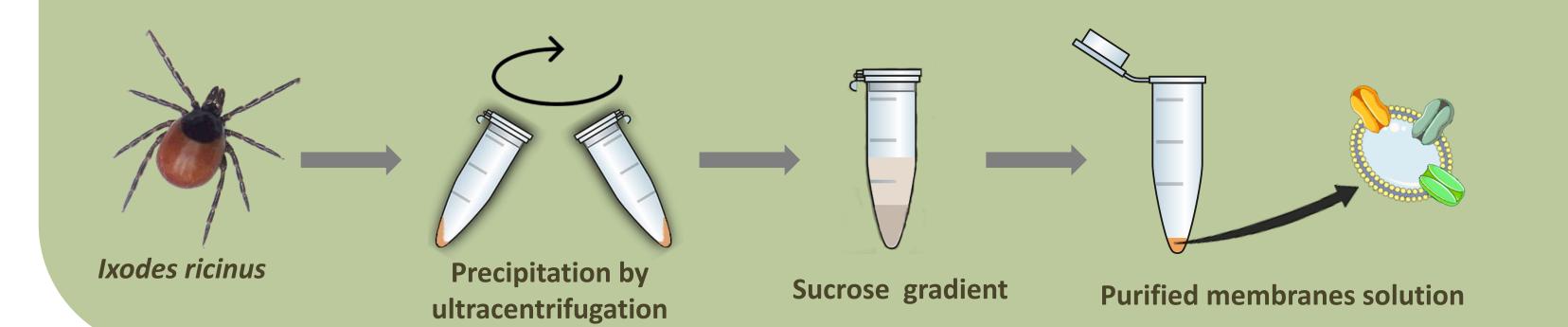
Introduction

Ticks are known as harmful parasites and vectors of many microorganisms that could be pathogenic for animal and human. For example, the castor bean tick, *Ixodes ricinus*, can transmit diseases like Lyme borreliosis and tick-borne encephalitis, becoming a worldwide threat to public health (Boulanger *et al.*, 2019). Due to the lack of effective vaccines or other non-chemical based methods, acaricides remain the main way for tick control. However, there is now increasing evidence of resistance development against currently used acaricides (Kumar *et al.*, 2019). Thus, new compounds are needed and one strategy could be to target tick neuronal nicotinic acetylcholine receptors (nAChRs). In the present work, we aim to validate the membrane microtransplantation technique as a tool to study the pharmacological profiles of the tick nAChRs expressed in the synganglion. This technique allows to microtransplant the transmembrane receptors maintained in their natural lipidic environment. Thus, the studied native receptors keep their natural properties and regulation (Eusebi *et al.*, 2009).

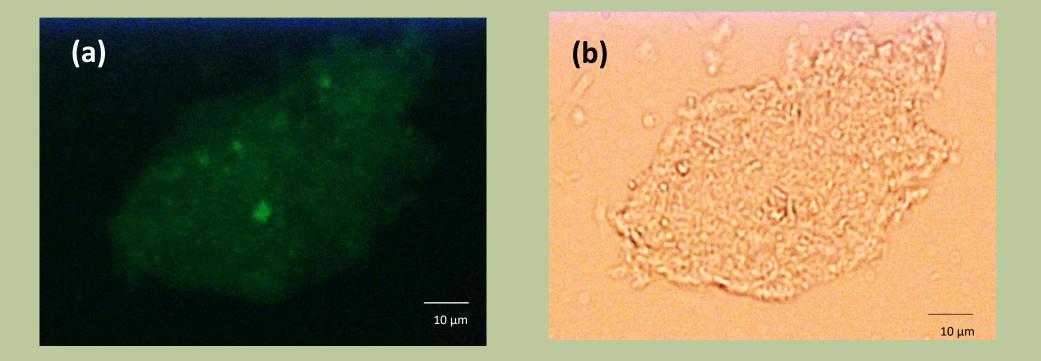
Extraction and purification of tick synganglion membranes

The membrane extraction and purification protocol consists in two crucial steps:

- The first is the dissociation of the neuronal tissues of *I. ricinus* (synganglion) and the precipitation of the transmembrane proteins by ultracentrifugation.
- The second is the purification of the membrane containing the transmembrane proteins of interest by using a sucrose density gradient.

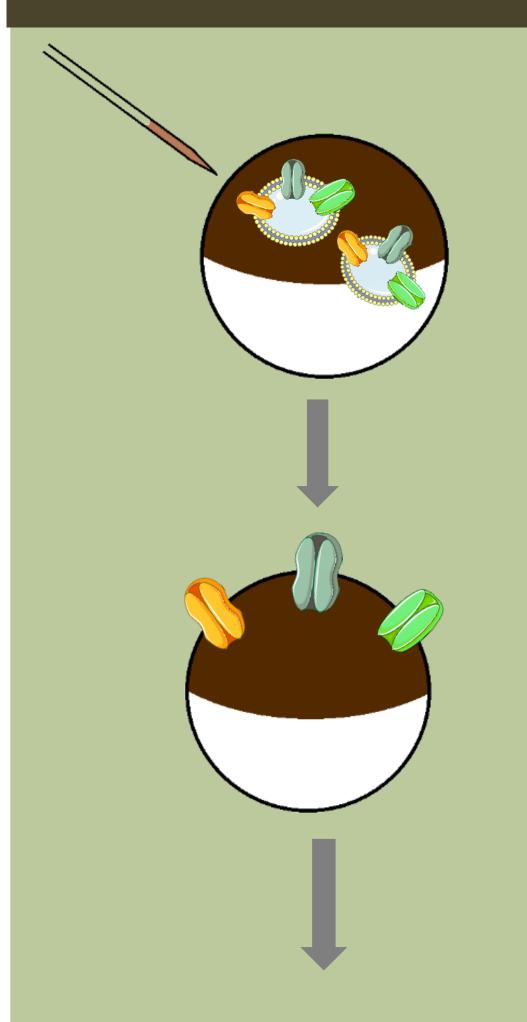


To validate the membrane preparation protocol we used α-bungarotoxin conjugated with Alexa Fluor[®]488 to label functional nAChRs.



Fluorenscent visualisation of the α -bungarotoxin-sensitive nAChRs using α bungarotoxin conjugated with Alexa Fluor®488 in membrane preparation from the tick *lxodes ricinus*. (a) membranes labeled with α -bungarotoxin conjugated with Alexa Fluor®488, each fluorescent spot highlighted the presence of functional receptors; (b) same preparation with transmitted light showing the membrane aggregates.

Electrophysiological recordings of nAChRs expressed on the oocyte membrane

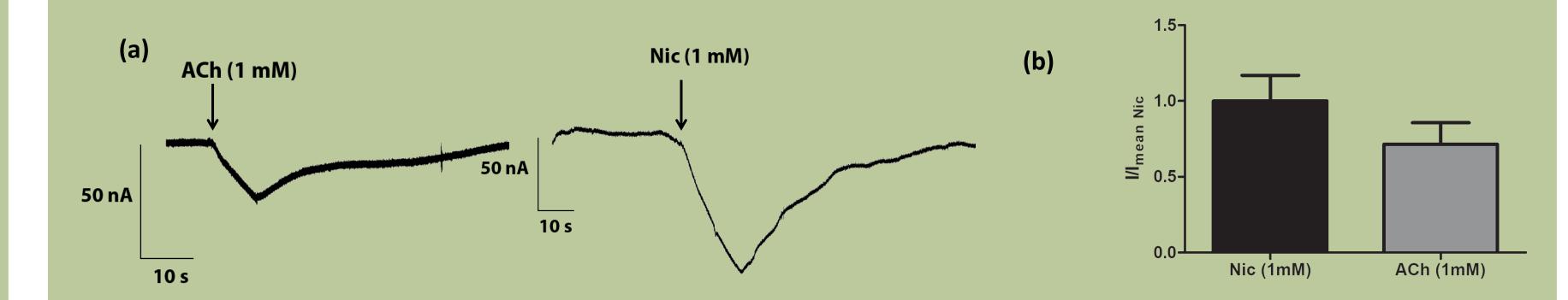


1 - Membrane injection

Injection of tick membrane

Native nAChRs of *Ixodes ricinus* are sensitive to currently used neonicotinoids





solution is made with a glass micropipette using a nanoinjector.

2 - Incubation

An incubation time of 12 to 24h, at 18°C, allows the recycling of tick membrane vesicles. At the end of this step native tick nAChRs are present at the oocyte membrane.

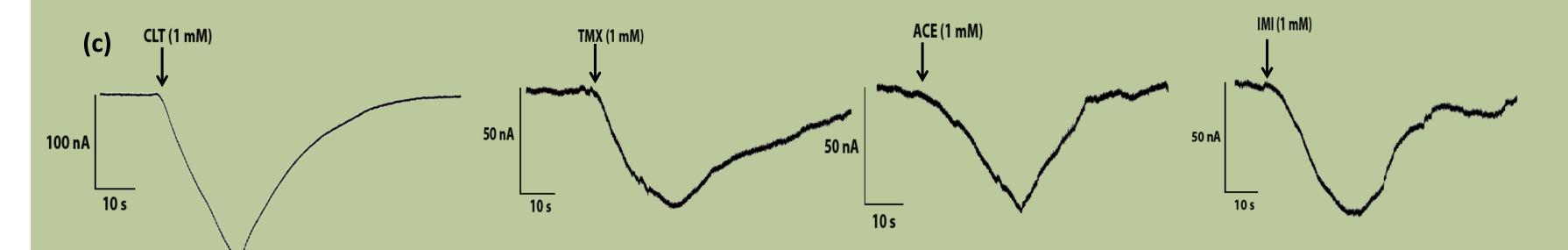
3 - Recordings

Injected oocytes are placed into an electrophysiology recording The two-electrode system. voltage-clamp (TEVC) technique setting is by used two Current microelectrodes on the oocyte detector membrane. One electrode clamp the membrane potential to -60 mV, the second one records the change in voltage after application of molecules. The response resulting from the opening of channels (response of the nAChRs) expressed on the oocyte membrane is recording.

(a) Example of currents obtained after application of ACh and Nic at 1 mM. (b) Effect of the ACh compared with the response induced by 1 mM Nic application; acetylcholine-induced current are smaller than nicotine-induced current (results are the mean of 13 independent experiments; error bars correspond to SEM).

We tested the activation of tick nAChRs from synganglion membrane preparation by acetylcholine (ACh) and nicotine (Nic). Both molecules are able to activate the receptors, and nAChRs are more sensitive to nicotine than to acetylcholine. These pharmacological properties are typical of nAChRs.

Native nAChRs are differently modulated by neonicotinoids according to molecules.



(c) Example of currents obtained by application of CLT, TMX, ACE, IMI at 1 mM. (d) Comparison of the maximum currents obtained with different neonicotinoids. Currents are normalised with the nicotine-induced current. Results are the mean of 6 to 9 independent experiments, error bars correspond to SEM.

We tested the receptor sensitivity to currently used neonicotinoids: clothianidin (CLT), thiametoxam (TMX), acetamiprid (ACE), imidacloprid (IMI).

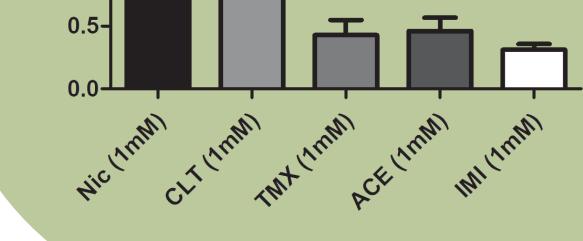


V_M ↑ Potential: -60mV

Recording of

membrane

potential



TMX, ACE, IMI induced similar currents that are lower than nicotine-induced current. On the contrary, CLT is able to induce higher current than those induced by Nic. These results show that tick native nAChRs are differently sensitive to neonicotinoids according to molecules.

Conclusion

- We validated and adapted the membrane microtransplantation protocol for the pharmacological characterisation of tick native nAChRs.
- We applied this technique for the screening of four neonicotinoids. Preliminary results highlighted that tick nAChRs are highly sensitive to clothianidin.

2.5-

2.0-

^{.0} Z 1.5-

^E 1.0-

(d)

• To complete this study, we aim to adapt this technique to other arthropod species and for the screening of other molecules.

This study gives promising results for the screening and selection of new anti-parasitic compounds targeting arthropod transmembrane proteins.

Boulanger N., Boyer P., Talagrand-Reboul E., Hansmann Y., (2019) Ticks and tick-borne diseases. Médecine et maladies infectieuses, 49, pp 87-97. Eusebi F., Palma E., Amici M., Miledi R., (2009) Microtransplantation of ligand-gated receptor-channels from fresh or frozen nervous tissue into Xenopus oocytes: A potent tool for expanding functional information. Progress in Neurobiology, 88, 32-40. Kumar R., (2019) Molecular markers and their application in the monitoring of acaricide resistance in *Rhipicephalus microplus*. Experimental and Applied Acarology, 78, pp 149-172.