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Perturbation of tyraminergic/octopaminergic function inhibits oviposition in the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*

Raquel Cossío-Bayúgar [a], Estefan Miranda-Miranda [a], Verónica Narváez Padilla [b], Francisco Olvera-Valencia [c], Enrique Reynaud [d,*]

[a] Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria, Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias INIFAP, Carr. Fed. Cuernavaca-Cuautla No. 8534, Jiutepec, Morelos, CP 62550, Mexico
[b] Facultad de Ciencias, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa, Cuernavaca, Morelos 62209, Mexico
[c] Departamento de ectoparásitos y dípteros Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria SAGARPA, Mexico
[d] Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Avenida Universidad, 2001, Apartado Postal 510–3, Cuernavaca 62210, Mexico

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ABSTRACT

The cattle tick *Rhipicephalus microplus*, is one of the most damaging livestock ectoparasites. Tropical tick infestation limits the introduction of high-yield bovine varieties because they do not have immunity to the diseases transmitted by these ectoparasites. This tick is usually controlled with chemical acaricides but their indiscriminate use has created resistant populations. The discovery of new molecules that can be used for tick control is urgent. Based on the knowledge that octopamine, a biogenic amine analog to epinephrine, is central to the regulation of oviposition in several studied arthropods and that an imbalance in octopamine release causes sterility in a *Drosophila* model. Tyramine, octopamine and epinastine and 83 adrenergic compounds classified by their effect in the vertebrate systems were screened for their ability to block oviposition in *Rhipicephalus microplus*. Of these molecules, we found that 10 alpha-agonists, 3 alpha-antagonists, 5 beta-adrenergic agonists, 7 beta-antagonists and Norepinephrine were able to inhibit oviposition in this tick at pharmacological concentrations. Surprisingly, tyramine appears to be more potent than octopamine. The probable physiological causes of this inhibition are discussed. Our results suggest that although there are alpha adrenergic-like receptors in the tick, they do not behave in a manner completely analogous to their vertebrate counterparts.

1. Introduction

The cattle tick *Rhipicephalus* (*Boophilus*) *microplus* is distributed in tropical and subtropical areas around the world and is considered the most important tick in terms of the economic losses that it causes to the cattle industry. This livestock pest is also the leading carrier of diseases such as *anaplasmosis* and *babesiosis*. The basic strategy to prevent and control ticks and their transmitted diseases in the cattle industry is chemical treatment with several acaricide formulae (de Castro, 1997). Numerous reports identify a consistent increase in acaricide-resistant tick strains worldwide, including ticks that are resistant to pyrethroids, organophosphates, amidines and amitraz (Chen et al., 2007; George et al., 2004; Jonsson and Hope, 2007; Li et al., 2007, 2004). For these reasons, the discovery of new molecules or their derivatives that could be used for tick control is urgent.

In vertebrates, the adrenergic system controls a wide range of phenomena. It is mostly known as the trigger of the "fight or flight" response, but it also modulates heart rate, smooth and striated muscle contractile ability, lipolysis and neural excitability, and it modulates the energetic balance of the cell. The equivalent of the adrenergic system in invertebrates is the tyramine/octopamine system, which seems to control the very same phenomena with similar mechanisms (Hunt, 2007; Roeder, 2005). The equivalent of epinephrine in insects, arachnids, octopods and other invertebrates appears to be octopamine (OA). OA is considered to be a neurotransmitter, neurohormone and neuromodulator and controls many energy-demanding processes and behaviors, such as the following: egg laying, aggressive behaviors, feeding rates and defensive jumping. Its counterpart is tyramine (TA), which just as in the vertebrate epinephrine-norepinephrine system is either an agonist or an antagonist of OA depending on the concentration and on the receptor to which it is binding (Roeder, 2005; Roeder et al., 2003). In *Drosophila melanogaster*, a deficiency in OA affects memory consolidation, makes the flies more sensitive to alcohol and induces sterility in female flies due to an inability to lay eggs.

* Corresponding author. Address: Enrique Reynaud, Instituto de Biotecnología, UNAM, A.P. 510-3, Cuernavaca, Morelos 62210, México, Mexico.
E-mail address: enrique@ibt.unam.mx (E. Reynaud).

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Table 1
Beta-adrenergic compounds that inhibit *R. microplus* egg deposition.

Beta-adrenergic			
Substance	Effective at 10 μM	Effective at 50 μM	Effect
(±)-Isoproterenol HCl	*	–	Agonist beta-1, beta-2, increases heart rate
Alprenolol HCl	*	–	Non selective beta-blocker, inhibits vasoconstriction
CGP 12177 HCl	–	*	Beta-1 agonist, beta-2 selective antagonist
CGP 20712A methanesulfonate	–	*	Beta-1 blocker
ICI 89406	–	*	Beta blocker
Clenbuterol	–	*	Beta-2 agonist
Pindolol	*	–	Non selective beta blocker, beta agonist at high concentrations
Propranolol glycol	*	–	Non selective beta blocker, may have alpha-1 agonist activity
R-(+)-propranolol	–	*	Non selective beta blocker, partial alpha agonist
Salbutamol sulfate	*	–	Beta-2 agonist, smooth muscle relaxant
Sotalol HCl	*	*	Non selective beta blocker
ZD 7114 HCl	*	*	Beta-3 agonist

Asterisks denote concentrations at which compounds were significantly active ($p < 0.05$).

Table 2
Alpha-Adrenergic compounds that inhibit *R. microplus* egg deposition.

Alpha-adrenergic			
Substance	Effective at 10 μM	Effective at 50 μM	Effect
2-[[b-(4-Hydroxyphenol)ethyl]aminoethyl]-1-tetralone	*	–	Alpha-1-noradrenergic antagonista
3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]-1,5-dimethylpyrimido[5,4-B]indole-2,4-dione	–	*	Alpha 1 ligand
Chloroethylclonidine 2HCl	*	–	Irreversible agonist for adreno-receptors, in particular alpha1B, D, C and alpha2A/D-subtypes
Clonidine HCl	*	*	Alpha-2 adrenergic agonista
Dihydroergocristine mesylate	*	*	5-HT receptor antagonist; partial agonist at adrenergic and dopaminergic receptors.
Guanabenz acetate	*	–	Alpha-2 adrenergic agonist
Idazoxan	–	*	Alpha-2 adrenergic receptor antagonist, imidazoline receptor antagonist
L-(-)-Norepinephrine-(+)-bitartrate	*	–	Binds to all adrenergic receptors but beta-2
Naphazoline HCl	*	–	Alpha adrenergic agonist
Prazosin HCl	–	*	Alpha-2/imidazoline receptor agonist
Rilmenidine hemifumarate	*	–	Alpha-2/imidazoline receptor agonist
Tyramine	*	*	Alpha adrenergic agonist
Xylazine HCl	*	–	Alpha-2 adrenergic agonist

Asterisks denote concentrations at which compounds were significantly active ($p < 0.05$).

The egg-laying deficiency in this organism is primarily due to the deregulation of the contractile ability of the oviducts smooth muscle (Osborne, 1996; Rodriguez-Valentin et al., 2006; Roeder, 1999, 2005; Roeder et al., 2003). The receptor for OA in the *Drosophila* oviduct is of the OAMB type (Lee et al., 2003). OA induces oviduct smooth muscle relaxation through the cyclic AMP (cAMP) pathway (Rodriguez-Valentin et al., 2006). OA receptors are coupled to small G proteins (G_s) that are in turn coupled to adenylate cyclases. The stimulation of this kind of receptor augments the intracellular levels of cAMP, thus inducing smooth muscle relaxation through the protein kinase A (PKA) pathway, which affects Ca^{2+} levels (Anderson, 2006). The delicate balance between OA and its functional antagonist TA are critical for adequate response of the arthropod the environment. Egg deposition in arthropods is particularly sensitive to any imbalance in the relative concentration of these biogenic amines (Yamane and Miyatake, 2010). When the OA/TA system is imbalanced, oviposition is inhibited (Roeder, 2005; Roeder et al., 2003). It has been shown that some adrenergic agonists and antagonists have a higher affinity for the arthropod receptors than for their mammalian counterparts (Roeder et al., 1995, 1998). This differential affinity provides the opportunity to identify new molecules that could be used for tick control. Once these molecules are identified, they could be used as such or used as "seeds" for further chemical modification to achieve even higher affinities or activities. Knowing these facts, we reasoned that there was a possibility that adrenergic agonists and antagonists could unbalance the levels of cAMP or Ca^{2+} homeostasis in the *R. microplus* oviduct and thus disrupt oviposition.

2. Materials and methods

2.1. Tick strain

An acaricide-susceptible reference strain was used in this study. This strain has been cultured under laboratory controlled conditions for many generations at the Departamento de Ectoparásitos y Dípteros del Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA-SAGARPA) in Mexico and used as a susceptible reference for the tick acaricide resistance monitoring programs of the Mexican federal government. The reference strain was obtained by infesting a bovine with 20, 000 10- to 15- day old larvae.

2.2. Oviposition assay

Engorged females were collected 21 days after infestation and placed in Petri dishes and then incubated at 28 °C in 80% relative moisture until oviposition. The ticks were injected with drugs on the 3rd day of oviposition using a 30 gauge syringe as described previously (Baier et al., 2002; Booth, 1989). This was performed