RESUMEN

El nematodo de vida libre *Caenorhabditis elegans* es un organismo modelo para el estudio del sistema nervioso y enfermedades humanas. Este nematodo del suelo ofrece un gran potencial para análisis genéticos, en parte debido a su rápido ciclo de vida (3-días), pequeño tamaño (1,5 mm de largo) y fácil cultivo en el laboratorio.

Progresos sustanciales en la identificación de genes que codifican para un gran número de proteínas responsables de la liberación de neurotransmisores, la detección postsináptica y las señales corriente abajo, han avanzado nuestro entendimiento de la mecanística de cómo las neuronas se comunican he interactúan.

Caenorhabditis elegans es también un valioso modelo para el estudio de agentes antihelmínticos relacionados al sistema nervioso. Los receptores "Cys-loop" musculares de nematodos son de importancia clínica porque son blancos de drogas antihelmínticas. Los nematodos parásitos causan sustanciales muertes y morbidez en humanos y pérdidas en el ganado y animales domésticos. *C. elegans* es entonces una valiosa plataforma para el estudio de blancos antihelmínticos porque comparte características fisiológicas y farmacológicas con los nematodos de vida parasitaria, y es sensible a la mayoría de las drogas antihelmínticas. También ha emergido como un organismo modelo útil para el estudio de enfermedades neuromusculares humanas y testeo de drogas.

En este trabajo de Tesis nosotros exploramos a diferentes niveles los receptores involucrados en la coordinación de la locomoción de los gusanos. En *C. elegans* los músculos de la pared del cuerpo reciben innervación de las neuronas motoras colinérgicas (excitatorias) y GABAérgicas (inhibitorias). La acetilcolina (ACh) liberada desde las neuronas motoras estimula la contracción muscular sobre un lado del cuerpo, y simultáneamente activa una neurona motora inhibitoria que se proyecta hacia el lado opuesto del cuerpo y libera GABA, el cual relaja los músculos. Debido a que *C. elegans* contiene un receptor de GABA y dos tipos farmacológicamente diferentes de receptores de ACh (AChR), el AChR sensible a levamisol (L-AChR) y el

AChR sensible a nicotina (N-AChR), decidimos dividir este trabajo en dos capítulos para una lectura más conveniente.

En el **Capítulo I**, exploramos los AChRs. El AChR es un miembro de la familia de receptores "Cys-loop", la cual media la transmisión sináptica rápida en vertebrados e invertebrados. AChR pueden ensamblarse de cinco subunidades tipo-α idénticas – formando receptores homoméricos – como el receptor de vertebrados α7 o el N-AChR de *C. elegans* (ACR-16) o de diferentes subunidades α y no-α formando receptores heteroméricos, como los nicotínicos musculares de vertebrados y los L-AChRs musculares de nematodos.

El músculo de *C. elegans* contiene siete diferentes subunidades de AChRs, cinco de las cuales han sido mostradas como componentes del L-AChR adulto. Para dilucidar la razón de tal diversidad de subunidades, exploramos sus roles funcionales en células musculares de la Larva 1 (L1). Por medio de ensayos de canal único y corrientes macroscópicas demostramos que las subunidades tipo-α UNC-38 y UNC-63 como la no-α UNC-29 son requeridas para L-AChRs funcionales. Asimismo exploramos en detalle la contribución de las subunidad tipo-α LEV-8 y ACR-8.

Nuestro estudio revela que la subunidad LEV-8 es un componente del L-AChR nativo en L1 pero se comporta como una subunidad no esencial. Esta juega un rol clave en el mantenimiento de una baja velocidad y extendido de la desensibilización de los L-AChRs. También mostramos que en ausencia de la subunidad tipo-α ACR-8, la propiedades de los canales de L-AChRs no son modificadas, por lo tanto indicando que ACR-8 no es un componente del L-AChR en L1. Este estudio revela que las células L1 expresan un tipo principal de L-AChR compuesto de cinco diferentes subunidades: UNC-38, UNC-63, UNC-29, LEV-1, y LEV-8. El análisis de una doble mutante nula *lev-8;acr-8*, la cual muestra un fenotipo descoordinado y es resistente a levamisol, revela que ACR-8 puede reemplazar a LEV-8 en su ausencia, por lo tanto atribuyéndosele un rol a esta subunidad.

En el **Capítulo II**, exploramos el UNC-49R. Los canales de cloro activados por GABA juegan un importante rol inhibitorio en el sistema nervioso de vertebrados e invertebrados. El receptor muscular de GABA de *C. elegans* está codificado por el gen *unc-49*, el cual es traducido en tres subunidades: UNC-49A, UNC-49B y UNC-49C. Ha sido mostrado que en el estado adulto de *C. elegans* el receptor de GABA está compuesto de las subunidades B y C. Los UNC-49Rs comparten superposiciones estructurales y farmacológicas con los receptores $GABA_A$ de mamífero en algunos aspectos y difieren grandemente en otros. Estas diferencias podrían ser explotadas en el diseño de drogas antiparasíticas. Sin embargo, la información sobre las propiedades funcionales de los receptores de nematodos es todavía escasa.

Por medio de ensayos de corrientes macroscópicas y de canal único desciframos como los receptores de GABA de células musculares al estadio L1 de *C. elegans* son activados y modulados por agonistas y agentes antihelmínticos. Mostramos que muscimol, el cual es un agonista selectivo de los $GABA_A$Rs, y piperazina, un antihelmíntico ampliamente utilizado, son ambos capaces de activar al UNC-49R. Los efectos de estas drogas a nivel molecular están relacionados con efectos comportamentales en ensayos de parálisis. Es interesante el hecho de que nuestros resultados revelan que la ivermectina (IVM), la cual es un modulador de muchos receptores "Cys-loop", inhibe los receptores de GABA como también los L-AChRs de *C. elegans*, ambos involucrados en el movimiento coordinado. Más aún, la IVM muestra efectos sinérgicos sobre la parálisis inducida por ambos agonistas GABAérgicos y colinérgicos. Por lo tanto, reforzando la importancia de la investigación sobre la combinación de drogas antihelmínticas como estrategia tendiendo a reducir el incremento de problemas de resistencia a drogas.

En general, nuestros estudios en el capítulo II proveen nueva información concerniente a la activación de los $GABA_A$Rs en el músculo de *C. elegans*. Mostramos por primera vez la actividad de canal único del UNC-49R nativo, como también que muscimol y piperazina son agonistas de este receptor. Nuestro estudio también provee más información sobre los complejos y pleiotrópicos efectos de la IVM. La IVM es un inhibidor de los receptores de GABA y L-AChR. La elucidación de las bases

estructurales y mecanísticas bajo las acciones pleiotrópicas de la IVM en la familia de receptores “Cys-loop” puede abrir puertas para el diseño de nuevas drogas.

En resumen, en esta Tesis doctoral caracterizamos el L-AChR y la activación del UNC-49R, ambos involucrados en la locomoción coordinada.

La caracterización de estos receptores “Cys-loop” en un organismo genéticamente manipulable y modelo de nematodos parásitos provee nuevas avenidas de exploración para drogas selectivas, como también para definir los determinantes estructurales de la activación y modulación en la familia de receptores “Cys-loop”.

SUMMARY

The free-living nematode *Caenorhabditis elegans* is a model organism to study the nervous system and human diseases. This soil nematode offers great potential for genetic analysis, partly because of its rapid (3-day) life cycle, small size (1.5-mm-long adult) and ease of laboratory culture. Substantial progress in the identification of genes encoding for a large number of proteins responsible for neurotransmitter release, postsynaptic detection and downstream signaling has advanced our understanding of the mechanisms by which neurons communicate and interact.

Caenorhabditis elegans is also a valuable model for the study of anthelmintic agents related to the nervous system. Nematode muscle Cys-loop receptors are of clinical importance because they are targets of anthelmintic drugs. Nematode parasites cause substantial mortality and morbidity in humans and losses in livestock and domestic animals*. C. elegans* is a valuable platform for the study of anthelmintic targets because it shares physiological and pharmacological characteristics with parasitic nematodes, and it is sensitive to most anthelmintic drugs. It has also emerged as a useful model organism for studying human neuromuscular diseases and for drug testing.

In this Thesis we have explored at different levels the receptors involved in worm coordinated locomotion. In *C. elegans*, the body wall muscles receive innervations from both cholinergic (excitatory) and GABAergic (inhibitory) motor neurons. Acetylcholine released from motor neurons stimulates muscle contraction on one side of the body, and simultaneously activates an inhibitory motor neuron that projects to the opposite side of the body to release GABA. Since muscle cells contain one GABA and two different pharmacological types of acetylcholine receptors (AChR), the levamisole-sensitive AChR (L-AChR) and the nicotine-sensitive AChR (N-AChR), we decided to divide this work into two chapters for a more suitable lecture.

In **Chapter I**, we explored AChRs. The AChR is a member of the Cys-loop receptor family, which mediates fast synaptic transmission in vertebrates and invertebrates. AChRs can assemble from five identical α-type subunits, forming

homomeric receptors, such as vertebrate α7 or *C. elegans* ACR-16 (N-AChR) or from different α and non-α subunits forming heteromeric receptors, such as vertebrate and *C. elegans* muscle L-AChRs.

Caenorhabditis elegans muscle contains seven different AChR subunits, five of which have been shown to be components of the adult levamisole-sensitive AChRs (L-AChRs). To elucidate the reason for such subunit diversity, we explored their functional roles in the larva 1 (L1) muscle cells. By single-channel and macroscopic current recordings we demonstrate that the α-type UNC-38 and UNC-63 as well as the non-α UNC-29 subunits are required for functional L-AChRs. We explored in detail the contribution of the α-type LEV-8 subunit.

Our study reveals that it is a component of native L1 L-AChRs but behaves as a non-essential subunit. It plays a key role in maintaining a low rate and extent of desensitization of L-AChRs. We also show that in the absence of the α-type ACR-8 subunit, L-AChR channel properties are not modified, thus indicating that ACR-8 is not a component of L1 L-AChRs. This study reveals that L1 muscle cells express a main L-AChR type composed of five different subunits: UNC-38, UNC-63, UNC-29, LEV-1, and LEV-8. The analysis of a double *lev-8; acr-8* null mutant, which shows an uncoordinated and levamisole-resistant phenotype, reveals that ACR-8 can replace LEV-8 in its absence, thus attributing a functional role to this subunit.

In **Chapter II**, we have explored the UNC-49R. The GABA-gated chloride channels play an important inhibitory role in the nervous system of vertebrates and invertebrates. The *C. elegans* muscle GABA receptor is encoded by the *unc-49* gene, which is translated into three subunits: UNC-49A, UNC-49B, and UNC-49C. In adult *C. elegans* the GABA receptor has been shown to be composed of B and C subunits. UNC-49 receptors share significant structural and pharmacological overlap with mammalian $GABA_A$ receptors in some aspects and differ greatly in others. These differences could be exploited in parasitic drug design. However, the information about functional properties of nematode receptors is still scarce.

By analyzing at the macroscopic and single-channel level we deciphered how GABA receptors from *C. elegans* L1 muscle cells are activated and modulated by

agonists and anthelmintic agents. We show that muscimol, which is a $GABA_AR$ selective agonist, and piperazine, a widely used anthelmintic, are both able to activate the UNC-49R. The effects of these drugs at the molecular level are related to behavioral effects in paralysis assays. Interestingly, our results reveal that ivermectin, which has been shown to modulate several Cys-loop receptors, inhibits *C. elegans* GABA receptors as well as L-AChRs, which are also involved in the coordinated movement. Moreover, ivermectin shows synergistic effects on the paralysis induced by both GABAergic and nicotinic agonists, thus reinforcing the importance of research on anthelmintic drug combinations as a strategy tending to reduce the increasing problem of drug resistance.

In general, our study from chapter II provides novel information regarding $GABA_A$ receptor activation in *C. elegans* muscle. It shows for the first time single-channel activity of UNC-49 native receptor, and reveals that muscimol and piperazine, a widely used anthelmintic agent, are agonists. Our study also provides further information about the complex and pleiotropic effects of IVM: IVM is an inhibitor of *C. elegans* GABAR and levamisole-sensitive AChRs. Elucidation of the structural and mechanistic bases underlying the pleiotropic actions of IVM at the Cys-loop receptor family may open doors for novel drug design.

In summary, in this doctoral thesis we characterized the L-AChR and the activation of the UNC-49R, both involved in the coordinated locomotion.

The characterization of these Cys-loop receptors in a genetically tractable organism and model of parasitic nematodes provides new avenues of exploration for selective drugs as well as for defining structural determinants of activation and modulation in Cys-loop receptor family.

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PUBLICACIONES

I. Publicaciones originadas durante el periodo de formación doctoral

1. "Contribution of Subunits to *C. elegans* Levamisole-Sensitive Nicotinic Receptor Function"

Hernando G., Bergé I., Rayes D. y Bouzat C. (2012). *Molecular Pharmacology* 82:550–560. *pISSN: 0026-895X.*

2. "A Cys-loop mutation in the *Caenorhabditis elegans* nicotinic receptor subunit UNC-63 impairs but does not abolish channel function"

Jones A.K., Rayes D., Al-Diwani A., Maynard T.P., Jones R., **Hernando G.**, Buckingham S.D., Bouzat C. y Sattelle D.B. (2011). *The Journal of Biol Chem.* 286(4):2550-8. *pISSN:0021-9258.*

3. "An ER-resident membrane protein complex regulates nicotinic acetylcholine receptor subunit composition at the synapse"

Almedom R.B., Liewald JF, **Hernando G.**, Schultheis C., Rayes D., Pan J., Schedletzky T., Hutter H., Bouzat C. y Gottschalk A. (2009). *EMBO Journal* 28(17):2636-49*. pISSN: 0261-4189.*

4. "Activation of Single Nicotinic Receptor Channels from *Caenorhabditis elegans* Muscle"

Rayes D., Flamini M., **Henando G.** y Bouzat C. (2007). *Molecular Pharmacology* 71:1407-1415. *pISSN: 0026-895X.*

II. Publicaciones en preparación

"Activation and Function of GABA receptors in *Caenorhabditis elegans* muscle cells"

Hernando G. y Bouzat C. (2012). *En proceso de escritura.*