# RESUMEN


En esta Tesis doctoral se profundizó en el estudio de la interacción lípido-receptor de acetilcolina nicotínico (AChR) en dos aspectos: por un lado, el mecanismo de inhibición de ácidos grasos libres (AGLs), antagonistas no competitivos del AChR, y por el otro, la ubicación del AChR en dominios líquido-ordenados ($L_o$) condicionada por dos características particulares de la membrana.

Con la finalidad de dilucidar el mecanismo de antagonismo de los AGLs sobre el AChR, se utilizaron AGLs con un doble enlace único en diferentes posiciones de una cadena acílica de 18 átomos de carbono. Estudios funcionales realizados con la técnica de *patch-clamp* han mostrado que solo el *cis*-6-18:1 y el *cis*-9-18:1 reducen la duración del estado de canal abierto del receptor, sugiriendo, por lo tanto, un mecanismo de bloqueo alostérico del canal. Mediante espectroscopía de fluorescencia se comprobó que todos los AGLs se ubican en la interfase lípido-AChR, quedando el *cis*-6-18:1 restringido a los sitios denominados sitios anulares, mientras que el resto de los AGLs ocupa también sitios no-anulares. Por otro lado, estudios de polarización de fluorescencia mostraron que el AGLs *cis*-9-18:1 es el que ocasiona el mayor desorden en la membrana. Se comprobó i) que todos los *cis*-AGLs generan cambios conformacionales del AChR a nivel transmembrana, ii) que los *cis*-9-18:1, *cis*-11-18:1 y *cis*-13-18:1 perturban al AChR en su estado de reposo e iii) que los *cis*-6-18:1 y *cis*-9-18:1 son los que causan una mayor perturbación del estado desensibilizado. De esta manera, la posición e isomería del ángulo de torsión de los AGLs insaturados serían un factor clave en el bloqueo del AChR, sugiriendo entonces que los AGLs con un único doble enlace y ubicados superficialmente en la membrana inhiben en forma directa la función del AChR, posiblemente al perturbar una secuencia aminoacídica

transmembrana involucrada en los cambios alostéricos necesarios para la apertura del canal iónico.

Se postula que en la membrana plasmática el AChR se encuentra en dominios lipídicos denominados "balsas" ("*rafts*"). Sin embargo, el AChR no muestra preferencia por dominios $L_o$ en sistemas modelo compuestos por esfingomielina (SM), colesterol (Col) y POPC (1:1:1), pero sí lo hace un segmento transmembrana (γM4) que exhibe mayor contacto con los lípidos. Es decir, su distribución no dependería exclusivamente de sus propiedades intrínsecas sino también de señales extrínsecas a la proteína. En este trabajo de Tesis se estudió la posible partición diferencial del AChR en los dominios $L_o$ en dos sistemas modelo diferentes en función de: a) la presencia de diferentes especies puras de SM en la membrana, y b) la existencia de asimetría transbicapa en el sistema modelo, mediante el agregado de SM de cerebro (bSM) en la hemicapa externa. Tanto la existencia de asimetría como la presencia de 16:0-SM o 18:0-SM, en comparación con las bSM y 24:1-SM, producen una partición preferencial del AChR en los dominios $L_o$. De este modo, la localización del AChR en estos dominios depende no solo de sus propiedades sino también de las características propias de la membrana en la que se encuentra.

Entender la interacción lípido-AChR es de gran importancia para determinar tratamientos que puedan mejorar o inhibir la función del AChR y tratar enfermedades que lo involucren.

# ABSTRACT

In this Ph. D. thesis the understanding of the lipid-niconitic acetylcholine receptor (AChR) interaction was furthered in two aspects, namely the inhibition mechanism of free fatty acids (FFA), non-competitive AChR antagonists, and AChR location in liquid-ordered ($L_o$) domains conditioned by two membrane characteristics.

To elucidate FFA's antagonism mechanism, FFA with only one double bond in different positions of an 18-carbon acyl chain were tested on AChR. Patch-clamp functional studies showed that only *cis*-6-18:1 and *cis*-9-18:1 reduce the duration of the AChR open state, thus suggesting an allosteric blocking mechanism. Fluorescence spectroscopy measurements demonstrated that all FFA locate in the AChR-lipid interfase, with *cis*-6-18:1 restricted to anular sites, while the rest of the FFA tested also ocupy non-anular sites. Fluorescence polarization studies showed that *cis*-9-18:1 causes the highest membrane disorder of all FFA tested. It was determined that i) all *cis*-FFA generate AChR conformational changes at a transmembrane level, ii) only *cis*-9-18:1, *cis*-11-18:1 and *cis*-13-18:1 disturb AChR resting state and iii) *cis*-6-18:1 and *cis*-9-18:1 are the ones that cause the highest disturbance of the desensitized state. Thus, the position and isomerism of the torsion angle of unsaturated FFAs are probably a key factor in terms of AChR blockage, possibly by perturbing a transmembrane aminoacidic sequence involved in the allosteric changes necessary for ion channel gating.

In the plasma membrane, AChR is postulated to be located in lipid domains known as rafts. However, AChR shows no preference for $L_o$ domains in model systems – composed of sphingomyelin (SM), cholesterol (Chol) and POPC (1:1:1) –, but a transmembrane segment (γM4) that in closest contact with lipids does have

preference for them. This means that AChR distribution seems not to exclusively depend on its intrinsic properties but on signals external to the protein. In this Ph. D. thesis a posible differential AChR partitioning in $L_o$ domains was studied in two model systems as a function of a) the presence of different pure SM species in the membrane and b) the existence of transbilayer asymmetry in the model system, by the addition of brain SM (bSM) in the external hemilayer. Both asymmetry and the presence of either 16:0-SM or 18:0-SM, in comparison with bSM or 24:1-SM, lead to an AChR preferential partitioning in $L_o$ domains. AChR location in these domains depends not only on its properties but also on the characteristics of the membrane in which the ion channel is immersed.

Understanding lipid-AChR interaction is of great importance to determine treatments that can either improve or inhibit AChR function and, this, in turn, will help determining the treatment of diseases in which AChR is involved.

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