Influencia de Campos Electromagnéticos en las Propiedades Cinéticas de Canales Iónicos activados por Ligando

Resumen

En la comunicación neuronal, las transmisiones sinápticas son mediadas por canales iónicos activados por ligandos (LGIC), receptores que intervienen en procesos fisiológicos claves en el sistema nervioso central. La función esencial de estos receptores es acoplar la unión del neurotransmisor a la apertura del canal. Dado su papel esencial en la transmisión sináptica, los LGICs son blancos de agentes farmacológicos y numerosas patologías se asocian a su mal funcionamiento de estos receptores. Dentro de esta superfamilia de LGIC se encuentra la llamada familia de receptores *Cys-loop* que incluye a los receptores excitatorios nicotínico (AChR), de serotonina 5-HT_3 y receptores inhibitorios $GABA_A$ y de glicina.

Desde hace un tiempo, se ha discutido la posibilidad de que los campos magnéticos estáticos (CME) o electromagnéticos (CEM) resulten dañinos para la salud. Debido a los rápidos avances en las tecnologías de comunicación, la población está cada vez más expuesta a campos magnéticos. Esto aumenta la preocupación sobre los potenciales efectos para la salud derivados de la exposición a los mismos. A nivel celular, se ha propuesto que los campos probablemente inician sus efectos a través de los canales iónicos.

En el presente trabajo de tesis doctoral hemos estudiado la influencia de campos magnéticos estáticos (CME) y campos electromagnéticos (CEM) sobre dos miembros de la familia de receptores *Cys-loop*, el receptor de acetilcolina muscular adulto (AChR) y el receptor de serotonina homopentamérico tipo 3A (5-HT_{3A}R). El AChR es considerado el receptor modelo, tanto estructural como funcional, para todos los miembros de esta familia.

En primer lugar, estudiamos los efectos de CME sobre el AChR. Encontramos que un CME de intensidad entre 80-180 mT, a temperatura ambiente, no induce cambios sobre las constantes macroscópicas, así como tampoco en las propiedades

cinéticas a nivel de canal único. Dado que las propiedades diamagnéticas de la membrana celular se modifican por encima de una temperatura crítica, se realizaron estudios electrofisiológicos en un rango de temperatura de 5 °C a 50 °C, donde encontramos nuevamente que los LGIC no son sensibles a CME a otras temperaturas.

En segundo lugar, caracterizamos la influencia del CEM sobre los AChR y 5-HT_{3A}R. Los ensayos electrofisiológicos de corrientes macroscópicas mostraron que la amplitud de corriente disminuye en función de la frecuencia del CEM aplicado. La constante de decaimiento temporal no resulta modificada, mientras que el tiempo de activación aumenta significativamente. A nivel de canal único, encontramos que la exposición a los CEM no afecta la amplitud ni las constantes cinéticas de apertura y cierre. Sin embargo la frecuencia de episodios de activación (*clusters*), disminuye en función de la frecuencia. Es decir, que la presencia del CEM induce un nuevo estado no conductor, resultando en la disminución del pico de corriente y de la frecuencia de eventos de activación de *clusters*.

Si bien los cambios cualitativos del CEM fueron equivalentes para AChR y 5-HT_{3A}R, el receptor 5-HT_{3A} mostró mayor sensibilidad a los efectos del campo.

El trabajo desarrollado en esta tesis revela que los CEM pueden modificar la actividad de LGIC y abre puerta para entender los mecanismos moleculares y bases estructurales por los cuales los CEM modifican éstos receptores.

Influence of Electromagnetic Fields on Kinetics Properties of Ligand-gated Ion Channels

Summary

In neuronal communication, synaptic transmission is mediated by Ligand-Gated Ion Channels (LGICs), which are involved in fundamental physiological processes in the central nervous system. The essential function of these receptors is to couple neurotransmitter binding to channel opening. Given its essential role in synaptic transmission, the LGICs are targets of pharmacological agents and many diseases are associated with their incorrect function. The *Cys-loop* receptor family belongs to the LGIC superfamily and it includes the excitatory receptors, nicotinic and serotonin 5-HT_3, and inhibitory receptors, $GABA_A$ and glycine receptors.

For a while, it has been discussed the possibility that static magnetic fields (SMF) or electromagnetic (EMF) result harmful to health. Due to the rapid advances in communication technologies, the public is increasingly exposed to magnetic fields. This has raised concern about potential health effects resulting from exposure to them. At the cellular level, it is has been proposed that magnetic fields probably initiate their effects through ion channels.

In the present thesis we studied the influence of static magnetic fields (SMF) and electromagnetic fields (EMF) on two members of the *Cys-loop* family receptors, the nicotinic acetylcholine receptor (AChR) and the homopentameric serotonin type 3A receptor (5-HT_{3A}R). The AChR has been the structural and functional model for all members of this family.

First, we studied the effects of SMF on the AChR. We found that a strong SMF of 80-180 mT, at room temperature, does not induce any changes on macroscopic currents response to the agonist or in the kinetic properties at the single channel level. Since diamagnetic properties of the cellular membrane are modified above a critical temperature, electrophysiological studies were carried out at a temperature range of 5

°C to 50 °C. Again, we found that the LGIC receptor is not sensitive to SMF at a range of temperatures.

Secondly, we characterized the influence of EMF on the AChR and 5-HT_{3A}R. The electrophysiological recordings of macroscopic currents showed that the amplitude of the current decreases as a function of the EMF frequency applied. The temporal decay constant is not modified, whereas the rise time increases significantly. At the single channel level, we found that the exposure to EMF does not affect the amplitude or channel kinetics. However, the frequency of activation episodes elicited by agonist (*cluster*) is reduced significantly. Thus, the reduction of the peak current together with the frequency of channel suggests that EMF induces and stabilizes a new closed, non conductive state. Receptors not affected, or leaving this state, do not show changes in activation kinetics.

The EMF affects the functionality of both AChR and 5-HT_{3A}R being this influence on the 5-HT_{3A}R is steeper that on the AChR.

The work in this thesis contributes to understand how the EMF can modify the activity of LGIC and obtain the molecular mechanisms by which EMFs alter the kinetics of these receptors.

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