Poster P.09.60

ALTERED L-TYPE CHANNEL GATING, ACTION POTENTIAL FIRING AND EXCITATORY/INHIBITORY SYNAPTIC RESPONSES IN HIPPOCAMPAL NEURONS OF THE AUTISTIC TIMOTHY SYNDROME TYPE-2 MOUSE

Calorio C.[1], Hidisoglu E.[2], Chiantia G.[1], Gavello D.[1], Salio C.[1], Sassoè--Pognetto M.[1], Defilippi P.[1], Balzac F.[1], Turco E.[1], Bett G.C.L.[3], Rasmusson R.L.[3], Marcantoni A.[1], Carbone E.*[1]

[1]University of Torino ~ Torino ~ Italy, [2]Akdeniz Üniversitesi ~ ANTALYA ~ Turkey, [3]Dept. of Physiol. & Biophysics, The State Univ. of New York ~ Buffalo NY ~ United States of America

Tymothy syndrome (TS) is a multisystem disorder featuring cardiac arrhythmias, autism and adrenal gland dysfunction that originates from a de novo point mutation in the gene encoding the Cav1.2 (CACNA1C) L-type channel [1, 2]. Using the autistic TS2-neo mouse bearing the G406R point mutation associated with TS type-2 [3], we have recently shown that the mutation reduces the rate of inactivation and shifts leftward the activation and inactivation of L-type channels, causing marked increase of resting Ca2+ influx ('window' Ca2+ current) of adrenal mouse chromaffin cells (MCCs). The increased 'window current' causes marked reduction of Nav channel density, switches normal tonic firing to abnormal burst firing, reduces mitochondrial metabolism, induces cell swelling and decreases catecholamine release. Overnight incubation with nifedipine restores Nav channel density, normal MCC firing and quantity of catecholamine released [4].
Here we report that in cultured hippocampal neurons (HNs; 3-10 days-in-vitro) of TS2-neo mutated mice [5], L-type calcium currents were less inactivated during pulses of 1 s to +10 mV. The voltage-dependence of activation and steady-state inactivation were both leftward shifted (-6 and -11 mV, respectively), as reported for MCCs. The shifts generated an increased resting window Ca2+ current. Immunolabeling of pyramidal and GABAergic neurons indicated a 15% loss of pyramidal neurons and a 8% loss of interneurons in TS2-neo cultures compared to WT. Current-clamp data analysis indicated the existence of two groups of neurons: a "slow-spiking" that was predominant in WT cultures and a "fast-spiking" that was predominant in TS2 cultures. The TS2-mutation increased the mean firing frequency of both groups but reduced to about 50% the number of HNs able to fire more than two APs, even under sustained depolarization.
In pharmacologically isolated GABAergic mono-synapses of 12-18 DIV [6], the amplitude of electrically-induced IPSCs increased by 72% and the pair-pulse depression increased by 39% at 25 ms pulse separation in mutated HNs. In isolated glutamatergic neurons of 15-18 DIV forming self-synapses (autapses) [7], the electrically evoked EPSCs associated to AMPA receptors increased by 74% in mutated neurons. Peak-variance fluctuation analysis (PVFA) [8] of mEPSCs shows that the increased amplitude of EPSCs could be partially due to a 56% increase of single AMPA receptors conductance and not to an increased number of expressed AMPA receptors. In conclusion, the L-type channel gating changes induced by the TS2-type mutation of CACNA1C gene is most likely responsible for the increased resting window Ca2+ current, which results in reduced neuronal viability, altered excitability and "gain of function" of both GABAergic and glutamatergic synaptic responses.

La sindrome di Tymothy (TS) è un disordine multisistemico che si manifesta attraverso aritmie cardiache, autismo e disfunzioni della ghiandola surrenale. La sindrome origina da una mutazione de novo del gene CACNA1C che codifica per il canale del calcio Cav1.2 di tipo L [1, 2]. Utilizzando il topo autistico TS2-neo a cui è stata indotta la mutazione G406R associata alla TS di tipo 2 (TS2) [3], abbiamo dimostrato che la mutazione TS2 riduce l'inattivazione e sposta verso potenziali più negativi

la voltaggio dipendenza dei canali L, causando aumentati flussi di Ca2+ (“window current”) in cellule cromaffini di topo. L’aumentata “window current” causa riduzione della densità dei canali del sodio (Nav), induce “bursts” di potenziali d’azione (PA), riduce il metabolismo mitocondriale, induce rigonfiamento cellulare e diminuisce il rilascio di catecolamine. Trattamenti cronici con nifedipina per 18 ore ripristinano la densità dei Nav, la normale eccitabilità delle cellule e la quantità di catecolamine rilasciate [4].
Qui riportiamo che in neuroni ippocampali (NI) mantenuti in coltura per 3-10 giorni (DIV) del topo mutato TS2-neo, le correnti L sono meno inattivanti. Come nelle cellule cromaffini, la voltaggio dipendenza dell’attivazione e dell’inattivazione sono spostate a sinistra (-6 e -11 mV), causando un’aumentata “window current”. Il conteggio di neuroni piramidali e interneuroni inibitori immunomarcati per il GABA e il glutammato suggerisce una perdita del 15% di neuroni piramidali e dell’8% di interneuroni GABAergici in colture di NI TS2-neo mutati rispetto ai WT. Misure di PA in current-clamp suggeriscono l’esistenza di due gruppi di neuroni: un tipo “slow-spiking”, predominante in colture WT e un tipo “fast-spiking”, predominante in colture TS2-neo. La mutazione TS2 aumenta la frequenza media dei PA di entrambi i gruppi di neuroni ma riduce di circa il 50% il numero di neuroni in grado di generare più di due PA.
In preparati monosinaptici di neuroni GABAergici di 12-18 DIV [5], l’ampiezza degli IPSCs evocati elettricamente aumenta del 72% in neuroni mutati, mentre la depressione indotta da doppi impulsi aumenta del 39% quando i due impulsi sono separati da 25 ms. Parallelamente, in autapsi di neuroni glutamatergici isolati (15-18 DIV) [6], l’ampiezza degli EPSCs associati ai recettori AMPA aumenta del 74% in neuroni mutati. Un’analisi delle fluttuazioni del picco e della varianza degli mEPSCs [7], suggerisce che l’aumentata ampiezza degli EPSCs è verosimilmente dovuta ad un aumento della conduttanza dei recettori AMPA (56%) piuttosto che ad un aumento dei recettori AMPA espressi. In conclusione, i cambi delle proprietà cinetiche dei canali del calcio di tipo L indotte dalla mutazione TS2 sono responsabili dell’aumentata “window current” che causa un ridotto numero di neuroni funzionanti, un’alterata eccitabilità e un “gain-of-function” di entrambe le risposte sinaptiche GABAergiche e glutamatergiche in neuroni ippocampali in coltura.

1. Splawski I, Timothy KW, Decher N, Kumar P, Sachse FB, Beggs AH, et al. Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. Proceedings of the National Academy of Sciences of the United States of America 2005; 102:8089-96.

2. Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, et al. Ca(v)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. Cell 2004; 119:19-31.

3. Bader PL, Faizi M, Kim LH, Owen SF, Tadross MR, Alfa RW, et al. Mouse model of Timothy syndrome recapitulates triad of autistic traits. Proceedings of the National Academy of Sciences of the United States of America 2011; 108:15432-7.

4. Calorio C, Gavello D, Guarina L, Salio C, Sassoe-Pognetto M, Riganti C, et al. Impaired chromaffin cell excitability and exocytosis in autistic Timothy syndrome TS2-neo mouse rescued by L-type calcium channel blockers. The Journal of physiology 2019; 597:1705-33.

5. Gavello D, Rojo-Ruiz J, Marcantoni A, Franchino C, Carbone E, Carabelli V. Leptin Counteracts the Hypoxia-Induced Inhibition of Spontaneously Firing Hippocampal Neurons: A Microelectrode Array Study. Plos One 2012; 7.

6. Russo I, Gavello D, Menna E, Vandael D, Veglia C, Morello N, et al. p140Cap Regulates

GABAergic Synaptogenesis and Development of Hippocampal Inhibitory Circuits. Cerebral cortex (New York, NY : 1991) 2019; 29:91-105.

7. Ripoli C, Cocco S, Li Puma DD, Piacentini R, Mastrodonato A, Scala F, et al. Intracellular accumulation of amyloid-beta (Abeta) protein plays a major role in Abeta-induced alterations of glutamatergic synaptic transmission and plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience 2014; 34:12893-903.

8. Baldelli P, Hernandez-Guijo JM, Carabelli V, Carbone E. Brain-derived neurotrophic factor enhances GABA release probability and nonuniform distribution of N- and P/Q-type channels on release sites of hippocampal inhibitory synapses. Journal of Neuroscience 2005; 25:3358-68.

Sindrome di Tymothy

Coordinator: Emilio Carbone
Duration (N. Years): 3
Starting year: 2015

Telethon Project (nr):

GGP15110

Disease Name:

Tymothy Syndrome

Keywords:

Autism, L-type calcium channels, GABAergic glutamatergic synapsis