



Pannexin 1 restricts dendritic branching and formation of dendritic spines in hippocampal neurons: possible role of small Rho GTPases and F-actin.

Tesis entregada a
LA UNIVERSIDAD DE VALPARAÍSO
en Cumplimiento Parcial de los requisitos para optar al grado de
Doctor en Ciencias con Mención en Neurociencia
Facultad De Ciencias

Por
Carolina Andrea Flores Muñoz
Dirigida por: Dr. Álvaro O. Ardiles Araya
Co-Dirigida por: Dr. Agustín D. Martínez Carrasco

INDEX

<i>ACKNOWLEDGMENT</i>	<i>iv</i>
<i>FINANCIAL SUPPORT</i>	<i>v</i>
<i>INDEX</i>	<i>vi</i>
<i>LIST OF TABLES</i>	<i>ix</i>
<i>LIST OF FIGURES</i>	<i>x</i>
<i>ABBREVIATIONS OR NOMENCLATURE</i>	<i>xii</i>
<i>ABSTRACT</i>	<i>xv</i>
<i>INTRODUCTION</i>	<i>1</i>
An Overview of Pannexins	1
Activation of Panx1 Channels	2
Panx1 Expression in the CNS	3
Physiological Roles of Panx1	4
Panx1 and Neuronal Cytoskeleton	5
Panx1 and Structural Plasticity	5
Structural Organization of Actin Filaments in the CNS	6
Small Rho GTPases and Regulation	7
Small Rho GTPases and Neuronal Cytoskeleton	9
1. <i>Ras-related C3 botulinum toxin substrate 1 (Rac1)</i>	9
2. <i>Cell division cycle 42 (Cdc42)</i>	9
3. <i>Ras homologous member A (RhoA)</i>	10
Regulation of Rho GTPase signaling pathways at synapses	10
<i>HYPOTHESIS</i>	<i>13</i>
<i>GENERAL AIM</i>	<i>13</i>
<i>SPECIFIC AIMS</i>	<i>14</i>
<i>MATERIALS AND METHODS</i>	<i>15</i>

1. <i>Animals</i>	15
2. <i>Primary Mouse Hippocampal Neuron Culture</i>	15
3. <i>Reduction of Panx1 Expression by siRNA</i>	15
4. <i>gly-LTP in Cultured Hippocampal Neurons</i>	16
5. <i>Extracellular ATP Measurements</i>	16
6. <i>Morphological and F-actin Analysis In Vitro</i>	16
7. <i>Single-Neuron Cytosolic Calcium Measurements</i>	18
8. <i>Slice Preparation for Biochemical Experiments</i>	18
9. <i>Field Electrophysiological Recordings in Hippocampal Slices</i>	19
10. <i>Determination of Relative Amount of Synaptic Proteins</i>	19
11. <i>Rho GTPase Activity Assay</i>	20
12. <i>F-actin Quantification In Vivo</i>	21
13. <i>Golgi-Cox Staining</i>	21
14. <i>Dendritic Morphological Analysis</i>	21
15. <i>Dendritic Spines Morphological Analysis</i>	22
16. <i>Statistical Analysis</i>	22
RESULTS _____	24
Blockade or reduced expression of Panx1 channels enhanced dendritic arborization complexity of hippocampal neurons _____	24
Reduction or blockage of Panx1 channels enhances dendritic spines density of hippocampal neurons _____	30
Reduction or blockade of Panx1 channels enhance the content of F-actin in dendrites and dendritic spines _____	33
Blockade or reduced expression of Panx1 channels increases PSD-95 and its colocalization with F-actin in dendritic spines of cultured hippocampal neurons ____	36
Panx1 channels deletion influences hippocampal neuronal morphology <i>in vivo</i> _____	43

Panx1 channels ablation or blockade enhance the spines density of hippocampal pyramidal neurons	46
Blockade or lack of Panx1 channels increased levels of synaptic proteins in hippocampal neurons	49
Lack or blockade of Panx1 channels increases content of F-actin and expression of ABPs	51
Panx1 channels may influence the steady-state activity levels of Rho GTPases	55
Blockade of Panx1 channels regulate dendritic branching and density of dendritic spines by activating Rac1 and RhoA Rho GTPases	57
Panx1 channels could activate the Rho GTPase pathway via Ca²⁺/CaMKII-dependent signaling	69
<i>DISCUSSION</i>	73
<i>Summary of findings</i>	73
<i>Panx1 channels regulate neuronal structural modifications</i>	73
<i>Panx1 channels modulates the content of F-actin by ABPs</i>	76
<i>Panx1 regulates morphological changes by crosstalk between RhoA and Rac1 GTPases</i>	77
<i>Panx1 channels could activate the RhoA and Rac1 pathways via Ca²⁺?</i>	79
<i>Panx1 channels a new modulator of astrocytes processes and dendritic arborization in the hippocampus?</i>	81
<i>It is necessary to study Panx1 channels in neuronal morphology?</i>	82
<i>SUPPLEMENTARY MATERIAL</i>	85
<i>BIBLIOGRAPHY</i>	104

LIST OF TABLES

Table 1. Dendritic spine parameters <i>in vitro</i> resting conditions _____	30
Table 2. Dendritic spine classification parameters <i>in vitro</i> gly-LTP treated neurons _____	33
Table 3. Dendritic spine parameters <i>in vitro</i> gly-LTP treated neurons with DL-APV _____	42
Table 4. Dendritic spine classification parameters <i>in vivo</i> resting conditions _____	46
Table 5. Dendritic spine classification parameters <i>in vivo</i> gly-LTP treated neurons _____	49
Table 6. Dendritic spine classification parameters <i>in vitro</i> gly-LTP treated neurons with activator or inhibitor Rho GTPases _____	65

LIST OF FIGURES

Figure 1. Schematic illustration of the topological structure of Panxs _____	1
Figure 2. Mechanism of Panx1 channels activation _____	2
Figure 3. Cellular distribution of Panx1 channels in the brain _____	3
Figure 4. The neuronal actin cytoskeleton and its regulation by external factors _____	7
Figure 5. Overview of Rho GTPase regulation _____	8
Figure 6. The signaling pathway from Rho GTPases to the actin cytoskeleton _____	10
Figure 7. Regulation of Rho GTPase activity by synaptic activation _____	11
Figure 8. Control efficiency of Panx1 knockdown _____	25
Figure 9. ATP release depends on Panx1 channels activity in cultured hippocampal neurons __	25
Figure 10. Blockade or reduced expression of Panx1 channels increases dendritic length and branching of resting hippocampal neurons _____	27
Figure 11. Blockade or reduced expression of Panx1 channels results in increased spine density and F-actin content in resting conditions _____	29
Figure 12. Reduction or blockade of Panx1 channels results in increased spine density <i>in vitro</i> after gly-LTP induction _____	31
Figure 13. Panx1 channels reduction and blockade promoted an additional increase in dendritic spines density induced by gly-LTP _____	32
Figure 14. Reduction or blockade of Panx1 channels enhance F-actin content in dendrites and dendritic spines after gly-LTP induction _____	34
Figure 15. Blockade or reduced expression of Panx1 channels increase content of F-actin in dendrites and dendritic spines under resting or gly-LTP induction _____	35
Figure 16. Under resting conditions, reduction or blockade of Panx1 channels increase the PSD-95 puncta and colocalization with F-actin in cultured hippocampal neurons _____	37
Figure 17. Blockade or reduction of Panx1 increases the colocalization between F-actin and PSD-95 after gly-LTP induction. _____	38
Figure 18. Reduction of Panx1 increases the colocalization between F-actin and PSD-95 independent of gly-LTP induction _____	39
Figure 19. DL-APV blocks dendrites and spines growth induced by the blockade of Panx1 channels in hippocampal neurons _____	41
Figure 20. Enhanced dendritic arborization in Panx1 KO neurons under resting conditions _____	44

Figure 21. Enhanced spine maturation in Panx1 KO neurons under resting conditions	45
Figure 22. gly-LTP induced in hippocampal synapses of WT mice	47
Figure 23. Panx1 channels ablation or blockade enhanced the spine density of hippocampal pyramidal neurons after gly-LTP	48
Figure 24. Panx1 KO demonstrates alterations in pre-and postsynaptic proteins	50
Figure 25. Ablation or blockade of Panx1 channels changes synaptic protein composition after gly-LTP	51
Figure 26. Ablation or blockade of Panx1 channels increase F-actin content in pyramidal CA1 region	52
Figure 27. The lack of Panx1 channels increases the protein levels of ABPs under resting conditions	53
Figure 28. Lack or blockade of Panx1 channels increases the protein levels of ABPs after gly-LTP induction	54
Figure 29. Ablation or blockade of Panx1 channels affect RhoA and Rac1 GTPase activity	56
Figure 30. Blockade of Panx1 channels prevents the effect of the activation of RhoA signaling effects on dendritic complexity in hippocampal neurons	58
Figure 31. The changes in dendritic complexity by blockade of Panx1 channels are abolished by the inactivation of Rac1 signaling in hippocampal neurons	60
Figure 32. Panx1 channels blockade restored dendritic spines density by RhoA signaling pathway	62
Figure 33. Blockade of Panx1 channels increase spines density and colocalization between PSD-95 and F-actin by modulating RhoA and Rac1 signaling pathway	64
Figure 34. Panx1 channels modulate the activation of Rac1 and RhoA GTPase oppositely.	66
Figure 35. Blockade of Panx1 channels increases F-actin content in dendrites and dendritic by RhoA and Rac1 activity	68
Figure 36. Lack of Panx1 channels changes the expression of kinases involved in LTP induction	69
Figure 37. Blockade of Panx1 channels exhibit decrease in cytosolic Ca ²⁺ induced by gly-LTP	71
Figure 38. Proposed model for Panx1 channels under resting or gly-LTP conditions	84

The morphological integrity and plastic properties of neurons depend on the dynamics of the neuronal cytoskeleton and involve changes in synaptic morphology and electrical signaling. Dendrites and dendritic spines are the major locus for excitatory synapses, and the actin cytoskeleton is their principal structural and regulatory component. Hence, actin reorganization places a central role in regulating of dendritic arborization and dendritic spines formation and maturation. In this regard, the family of small Rho GTPases, RhoA, Rac1, and Cdc42 play an essential role in regulating structural plasticity by controlling the assembly and stability of the actin cytoskeleton. However, the signals that control the activation or inhibition of the different small Rho GTPases in neuronal development and plasticity are relatively unknown.

Pannexin 1 (Panx1) is a membrane protein that forms non-selective channels implicated in actin-dependent processes in neurons such as cell migration and neurite extension, suggesting that Panx1 also be involved in other structural changes such as those associated with synaptic plasticity.

Here, we investigate if Panx1 channels modulate F-actin remodeling-dependent structural plasticity in hippocampal neurons through a mechanism that involves small Rho GTPases activity. We observed that the absence or blockade of Panx1 channels upon resting conditions increased the length and complexity of the dendritic arbor of hippocampal neurons. Similarly, under the induction of long-term chemical potentiation by glycine stimulation, hippocampal neurons exhibited a higher dendritic spines density than control neurons.

Interestingly, the absence or blockade of Panx1 channels stimulated the content of F-actin and increased the expression and activity of Rac1 and Cdc42 Rho GTPases. Consistently, the inactivation of Rac1 prevents the effect of Panx1 channels inhibition on dendritic arborization and the density of dendritic spines.

Our results provide evidence that the role of Panx1 channels in neuronal morphology and structural synaptic plasticity relies on actin organization and dynamics by regulation of RhoA and Rac1 GTPase activity.