

**Aspects of  
Astrocytic Calcium Signaling:  
A Role for NAADP<sup>+</sup>  
and  
for Temperature**

Dissertation zur Erlangung des akademischen Grades des  
Doktors der Naturwissenschaften (Dr. rer. nat.)

eingereicht im Fachbereich Biologie, Chemie, Pharmazie  
der Freien Universität Berlin im Juli 2006

vorgelegt von

Dipl.-Biochem./ MSc. by Res. in the Life Sciences

Antje Cornelia Heidemann

geb. am 01.07.1976 in Nordenham

Gutachter: 1. Prof. Dr. Hans-Joachim Pflüger  
Freie Universität Berlin  
Institut für Biologie - Neurobiologie  
Königin-Luise-Str. 28-30  
14195 Berlin

2. Prof. Dr. Helmut Kettenmann  
Max Delbrück Centrum für Molekulare Medizin  
Zelluläre Neurowissenschaften  
Robert Rössle Str. 10  
13125 Berlin

Tag der Disputation: 16.11.2006

Danksagungen:

Ich möchte allen danken, die mir die Durchführung dieser Arbeit ermöglicht und mich dabei unterstützt haben, wie meinen Eltern, meiner Betreuerin Dr. Carola Schipke, meinen Kollegen, darunter im besonderen Dr. Oliver Peters, Brigitte Haas und Ulrike Pannasch und vor allem dem Leiter des Labors in dem die Arbeit ausgeführt wurde, Prof. Dr. Helmut Kettenmann. Desweiteren möchte ich mich bei den technischen Assistenten bedanken, im speziellen bei Christiane Gras und Irene Haupt für Ihre Hilfe bei Immunfärbungen und Zellkultur. Dank gilt auch Daniel Geissler für die Durchführung der HPLC Analyse.

Diese Arbeit wurde durch Drittmittel der Deutschen Forschungsgemeinschaft über den Sonderforschungsbereich 515 und ein Doktorandenstipendium des Graduiertenkollegs 238 finanziert.

---

## CONTENT

<b>1</b>	<b><i>INTRODUCTION</i></b> .....	<b>5</b>
<b>1.1</b>	<b>Glial cells – Astrocytes</b> .....	<b>5</b>
1.1.1	Role of astrocytes in the intact brain .....	6
1.1.2	Role of astrocytes in pathology - Reactive astrogliosis and swelling.....	8
<b>1.2</b>	<b>Ca<sup>2+</sup> signaling</b> .....	<b>9</b>
<b>1.3</b>	<b>Ca<sup>2+</sup> signaling in astrocytic communication</b> .....	<b>13</b>
1.3.1	Ca <sup>2+</sup> excitability of astrocytes .....	13
1.3.2	Ca <sup>2+</sup> -dependent astrocytic feedback on neuronal activity .....	15
1.3.3	Astrocytic intercellular Ca <sup>2+</sup> waves involved in long-range communication .....	16
1.3.4	“Spontaneous” Ca <sup>2+</sup> oscillations in astrocytes as a generator of neuronal activity .....	16
<b>1.4</b>	<b>NO in the CNS</b> .....	<b>18</b>
1.4.1	NO and Ca <sup>2+</sup> homeostasis.....	19
1.4.2	Mechanical strain-related NO signaling.....	21
<b>1.5</b>	<b>NAADP<sup>+</sup> and the CNS</b> .....	<b>21</b>
<b>1.6</b>	<b>Aims</b> .....	<b>24</b>
<b>2</b>	<b><i>MATERIAL AND METHODS</i></b> .....	<b>25</b>
<b>2.1</b>	<b>Animals</b> .....	<b>25</b>
<b>2.2</b>	<b>Preparation of acute brain slices</b> .....	<b>25</b>
2.2.1	Buffers and solutions.....	25
2.2.2	Preparation procedure .....	26
<b>2.3</b>	<b>Cell culture</b> .....	<b>27</b>
2.3.1	Solutions, buffers, culture media, and supplements .....	27
2.3.2	Glial culture preparations .....	28
2.3.3	Cerebellar neuron preparation.....	30
2.3.4	Cell lines .....	30
2.3.5	PLL coating.....	31
<b>2.4</b>	<b>Ca<sup>2+</sup> imaging</b> .....	<b>31</b>
2.4.1	Solvents.....	31
2.4.2	Agonist, antagonists, blockers.....	31

---

2.4.3	Fluo-4-loading of slices.....	33
2.4.4	Fluo-4-loading of cells in culture.....	34
2.4.5	Confocal- and Videomicroscopy.....	34
<b>2.5</b>	<b>Immunostaining.....</b>	<b>36</b>
2.5.1	Buffers.....	36
2.5.2	Antibodies.....	37
2.5.3	Perfusion fixation.....	37
2.5.4	Cryosections.....	38
2.5.5	Immunostaining of slices.....	38
2.5.6	Immunostaining of cultures.....	39
<b>2.6</b>	<b>Statistical analysis.....</b>	<b>40</b>
2.6.1	Reacting cell number.....	40
2.6.2	“Ca <sup>2+</sup> signaling activity” and “(overall) average amplitude change”.....	40
2.6.3	Statistical tests.....	41
<b>2.7</b>	<b>Manufacturers.....</b>	<b>41</b>
<b>3</b>	<b>RESULTS.....</b>	<b>43</b>
<b>3.1</b>	<b>NAADP<sup>+</sup>.....</b>	<b>43</b>
3.1.1	NAADP <sup>+</sup> induced different types of Ca <sup>2+</sup> responses in astrocytes.....	43
3.1.2	NAADP <sup>+</sup> repetitively induced Ca <sup>2+</sup> signaling in astrocytes.....	44
3.1.3	NAADP <sup>+</sup> acted directly on astrocytes.....	46
3.1.4	NAADP <sup>+</sup> precursors also induced Ca <sup>2+</sup> signaling in astrocytes.....	48
3.1.5	NAADP <sup>+</sup> -induced Ca <sup>2+</sup> signaling depended on functional connexin hemichannels/gap junctions and extracellular Ca <sup>2+</sup> .....	48
3.1.6	Ca <sup>2+</sup> influx is a component of NAADP <sup>+</sup> -induced Ca <sup>2+</sup> signals.....	49
3.1.7	NAADP <sup>+</sup> -induced Ca <sup>2+</sup> signaling depended on the integrity of intracellular Ca <sup>2+</sup> stores.....	51
3.1.8	Purinergic and adenosine-mediated contribution to the NAADP <sup>+</sup> -induced signal.....	53
3.1.9	Astrocytes and neurons expressed the NAADP <sup>+</sup> -synthesizing enzyme CD38 <i>in situ</i> .....	55
3.1.10	NAADP <sup>+</sup> triggered Ca <sup>2+</sup> signaling in cultured neurons and glial cells.....	56
<b>3.2</b>	<b>Stop of perfusion-induced Ca<sup>2+</sup> signaling.....</b>	<b>57</b>
3.2.1	Switching off the perfusion induced different types of Ca <sup>2+</sup> responses in astrocytes in acute cortical brain slices.....	57
3.2.2	Switching off the perfusion can repeatedly evoke Ca <sup>2+</sup> responses in astrocytes.....	59
3.2.3	Switching off the perfusion is accompanied by a drop in temperature, which is the major trigger of the observed Ca <sup>2+</sup> signalling.....	61
3.2.4	Basal Ca <sup>2+</sup> signaling activity in cortical astrocytes is higher at RT than at higher temperatures	

---

(30-33°C or 37-38°C).....	63
3.2.5 More cortical astrocytes exhibit Ca <sup>2+</sup> signaling at 30-33°C than at 37-38°C .....	67
3.2.6 The observed Ca <sup>2+</sup> signaling is mainly driven by Ca <sup>2+</sup> influx .....	69
3.2.7 Cellular swelling is involved in switching off the perfusion-evoked Ca <sup>2+</sup> signaling .....	70
3.2.8 Neuronal activity, connexin channels, glutamate, ATP, adenosine, and GABA do not contribute to the Ca <sup>2+</sup> signaling .....	72
3.2.9 NO is involved in switching off of the perfusion-evoked Ca <sup>2+</sup> signaling.....	73
<b>4 DISCUSSION.....</b>	<b>76</b>
<b>4.1 NAADP<sup>+</sup> .....</b>	<b>76</b>
4.1.1 NAADP <sup>+</sup> triggers Ca <sup>2+</sup> signaling in all major brain cell types .....	76
4.1.2 Question of specificity and contribution of the purinergic and adenosine-mediated pathway .....	77
4.1.3 NAADP <sup>+</sup> potentially enters the cell to act.....	78
4.1.4 NAADP <sup>+</sup> signaling requires normal extracellular Ca <sup>2+</sup> levels.....	78
4.1.5 NAADP <sup>+</sup> signaling requires both, lysosomes and thapsigargin-sensitive stores.....	79
4.1.6 Hypothetical mechanism of extracellularly applied NAADP <sup>+</sup> -action .....	80
<b>4.2 Stop of perfusion-induced Ca<sup>2+</sup> signaling.....</b>	<b>82</b>
4.2.1 Switching off of the perfusion induces Ca <sup>2+</sup> signaling which is caused by the decrease in temperature	82
4.2.2 The mechanism of hypothermia-induced Ca <sup>2+</sup> signaling .....	84
4.2.2.1 Ca <sup>2+</sup> -influx is mainly involved in hypothermia-induced Ca <sup>2+</sup> signaling.....	84
4.2.2.2 Hypothermia-induced swelling may contribute to the Ca <sup>2+</sup> signaling .....	85
4.2.2.3 The Ca <sup>2+</sup> signaling is not caused by neuronal activity or fluxes of substances through connexin channels.....	86
4.2.2.4 Hypothermia-mediated release of Ca <sup>2+</sup> elevating substances from the tissue – A role for NO ...	87
4.2.2.5 NO (SNOG) induces oscillatory Ca <sup>2+</sup> responses in astrocytes in acute cortical slices .....	88
4.2.2.6 Phototoxicity may contribute to Ca <sup>2+</sup> signaling at RT.....	89
4.2.3 Possible functions of hypothermia-induced Ca <sup>2+</sup> signaling.....	90
<b>5 SUMMARY.....</b>	<b>93</b>
<b>5.1 English summary .....</b>	<b>93</b>
<b>5.2 Deutsche Zusammenfassung .....</b>	<b>94</b>
<b>6 REFERENCES.....</b>	<b>96</b>
<b>7 ANHANG .....</b>	<b>131</b>
<b>7.1 ABBREVIATIONS .....</b>	<b>131</b>
<b>7.2 Lebenslauf.....</b>	<b>135</b>

---

<b>7.3</b>	<b>Publikationen .....</b>	<b>137</b>
7.3.1	Paper.....	137
7.3.2	Posterbeiträge.....	137
7.3.3	weitere besuchte Konferenzen/Symposien/Workshops.....	138
<b>7.4</b>	<b>Erklärung.....</b>	<b>139</b>