Aus der Klinik für Anästhesiologie m. S. operative Intensivmedizin

der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Genomic and non-genomic effects of mineralocorticoid receptors and glucocorticoid receptors and their roles of pain modulation

zur Erlangung des akademischen Grades

Doctormedicinae

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vorgelegt der Medizinischen Fakultät

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Abstract (English)

Corticosteroids, including mineralocorticoids and glucocorticoids, are best known for their effective relief of inflammation and pain through activation of the intracellular mineralocorticoid (MR) and glucocorticoid receptors (GR) that changes gene transcription in a classical genomic pathway. However in addition to these known genomic effects, corticosteroids have been recently shown to also elicit rapid non-genomic effects in the central nervous system. The exact localizationof MR and GR in a specific subset of sensory neurons and/or glia cells within the spinal cord and the peripheral nervous system as well as their potential in the modulation of sensory stimuli such as pain still remains elusive. In naïve rats as well as rats with FCA-induced hind-paw inflammation, I investigated the expression of MR and GR and their anatomical location in peripheral neurons and glia cells as well as their alterations under painful inflammatory conditions. Moreover, I examinedin behaviorexperiments changes in nociceptive mechanical sensitivity following the local and systemic application of MR or GR agonists with and without respective antagonists. Similar to kidney tissue, MR and GR mRNA and specific receptor proteins were identified in dorsal root ganglia. Double immunofluorescence confocal microscopy revealed that MR are predominantly expressed in unmyelinated peptidergic nociceptive neurons, whereas GR are prominent in both peptidergic and non-peptidergic nociceptive neurons. MR and GR expression in glia cells is not very prominent and seems to play a minor role. Local application (intraplantar or intrathecal) of the MR selective agonist aldosterone acutely increased, whereas MR selective antagonists reduced nociceptive behavior. Systemic injection of the GR agonist dexamethasone and the MR antagonist canreonate-K but less their combination dose-dependently attenuated nociceptive behavior during inflammatory pain. Overall, these studies provided firm evidence for the existence of MR and GR in specific nociceptive neurons and for hitherto unknown rapid non-genomic effects of MR and GR agonists/antagonists to modulate nociception.

Abstract (Deutsch)

Corticosteroide, einschließlich Mineralocorticoide und Glucocorticoide, sind am besten bekannt für ihre wirksame Linderung von Entzündung und Schmerz durch Aktivierung der intrazellulären Mineralocorticoid- (MR) und Glucocorticoid-Rezeptoren (GR), indem sie die Gentranskription im Sinne des klassischen genomischen Signalwegs verändern. Zusätzlich zu diesen bekannten genomischen Wirkungen wurde kürzlich gezeigt, dass Corticosteroide auch schnelle nicht-genomische Wirkungen im zentralen Nervensystem hervorrufen können. Bisher ungeklärt ist die genaue Lokalisation von MR und GR in spezifischen Untergruppen sensorischer Neurone und / oder Gliazellen innerhalb des Rückenmarks und peripheren Nervensystems sowie deren Potential bei der Modulation von Sinnesreizen, wie zum Beispiel Schmerz. In naiven Ratten sowie Ratten mit FCA-induzierter Hinterpfotenentzündung untersuchte ich die Expression von MR- und GR und deren anatomische Lokalisation in peripheren Neuronen und Gliazellen sowie deren Veränderungen unter schmerzhaften Entzündungsbedingungen. Darüber hinaus untersuchte ich in Verhaltensexperimenten Veränderungen der nozizeptiven mechanischen Sensitivität nach lokaler und systemischer Applikation von MR- oder GR-Agonisten mit und ohne respektive Antagonisten. Ähnlich wie im Nierengewebe konnten MR- und GR-mRNA und spezifische Rezeptorproteine in den Spinalganglien identifiziert werden. Die Doppelimmunfluoreszenz-Konfokalmikroskopie zeigte, dass MR vorwiegend in nicht-myelinisiertenpeptidergennozizeptiven Neuronen exprimiert werden, während GR sowohl in peptidergen als auch in nicht-peptidergennozizeptiven Neuronen vorherrschend sind. MR- und GR-Expression in Glia-Zellen war nicht sehr prominent und schien eine untergeordnete Rolle zu spielen. Die lokale Applikation (intraplantar oder intrathekal) des MR-selektiven Agonisten Aldosteron erhöhte, wohingegen die des MR-selektive Antagonisten reduzierte die nozizeptive mechanische Sensitivität. Systemische Applikation des GR-Agonisten Dexamethason und des MR-Antagonisten Canreonate-K, jedoch weniger ihre Kombination bewirkte eine dosisabhängige Reduktion des nozizeptiven Verhalten bei entzündlichen Schmerzen. Insgesamt lieferten diese Studien einen Beweis für die Existenz von MR und GR in spezifischen nozizeptiven Neuronen und für bisher unbekannte, schnell einsetzende nicht-genomische Wirkungen von MR- und GR-Agonisten / Antagonisten zur Modulation nozizeptiver Sinneswahrnehmungen.

Publications

1. Xiongjuan Li, Mohammed Shaqura,Doaa Mohamed, Antje Beyer, Shunji Yamada, Shaaban A. Mousa, Michael Schäfer. Pro- versus antinociceptivenongenomic effects of neuronal mineralocorticoid versus glucocorticoid receptors during inflammatory pain. **Anesthesiology2018** Jan 22. doi: 10.1097/ALN.0000000000002087.
[Epub ahead of print] PMID: 29356757,(IF: 5.66) TOP-Journal

2. Mohammed Shaqura, **Xiongjuan Li**, Mahmoud Al-Khrasani, Mehdi Shakibaei, SaschaTafelski, Susanna Fürst, Antje Beyer, Mitsuhiro Kawata, Michael Schäfer, Shaaban A. Mousa. Membrane-bound glucocorticoid receptors on distinct nociceptive neurons as potential targets for pain control through rapid non-genomic effects.
Neuropharmacology 2016, 111: 1-13,(IF: 5.012)

3. Shaqura, **Xiongjuan Li**, Mohammed A. Al-Madol, SaschaTafelski, Antje Beyer-Koczorek, Shaaban A. Mousa, Michael Schäfer. Acute mechanicalsensitization of peripheral nociceptors by aldosterone through non-genomic activation ofmembrane bound mineralocorticoid receptors in naive rats.
Neuropharmacology 2016, 107: 251-261, (IF: 5.012)

Declaration of individual contribution to the following publication:

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Xiongjuan Li contributed to the concept and study protocol of all behavioral experiments. She prepared the animals for the experiments, administered all required drugs, and performed all nociceptive animal testings. In addition, she participated in sacrificing the animals to obtain tissue of spinal cord and dorsal root ganglia. She contributed in the immunohistochemical and western blot experiments. She analyzed the respective results obtained from her experiments, performed the statistical tests, and wrote part of the methods and results section. Finally she contributed to the introduction and discussion of the manuscript, revised the final draft and approved the final version of the manuscript.

Unterschrift, Datum und Stempel des betreuenden Hochschullehrers

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Prof. Dr. med. Michael Schäfer

Unterschrift des Doktoranden

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Xiongjuan Li

Journal Data Filtered By: **Selected JCR Year: 2016** Selected Editions: SCIE,SSCI
Selected Categories: **"ANESTHESIOLOGY"** Selected Category Scheme: WoS
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Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	BRITISH JOURNAL OF ANAESTHESIA	19,005	6.238	0.028470
2	ANESTHESIOLOGY	28,232	5.660	0.031550
3	PAIN	35,333	5.445	0.044460
4	ANAESTHESIA	9,392	4.741	0.009600
5	ANESTHESIA AND ANALGESIA	25,375	4.014	0.023190
6	JOURNAL OF NEUROSURGICAL ANESTHESIOLOGY	1,469	3.925	0.002170
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8	REGIONAL ANESTHESIA AND PAIN MEDICINE	4,440	3.515	0.005550
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11	Pain Physician	3,431	2.840	0.007700
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14	ACTA ANAESTHESIOLOGICA SCANDINAVICA	7,021	2.438	0.008730
15	Current Opinion in Anesthesiology	2,557	2.369	0.004430
16	Canadian Journal of Anesthesia-Journal canadien d anesthesie	5,286	2.312	0.005410
17	PEDIATRIC ANESTHESIA	4,406	2.254	0.006450
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19	JOURNAL OF CLINICAL MONITORING AND COMPUTING	1,388	2.178	0.002530
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21	JOURNAL OF CARDIOTHORACIC AND VASCULAR ANESTHESIA	3,863	1.699	0.007030
22	ANAESTHESIA AND INTENSIVE CARE	2,663	1.695	0.003440
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Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
25	BMC Anesthesiology	867	1.525	0.002910
26	Journal of Anesthesia	1,813	1.399	0.003740
27	SCHMERZ	732	1.336	0.001130
28	ANNALES FRANCAISES D ANESTHESIE ET DE REANIMATION	989	1.131	0.001530
29	ANAESTHESIST	1,323	1.039	0.001270
30	Revista Brasileira de Anestesiologia	722	0.903	0.001040
31	ANASTHESIOLOGIE INTENSIVMEDIZIN NOTFALLMEDIZIN SCHMERZTHERAPIE	344	0.367	0.000290

Xiongjuan Li, Mohammed Shaqura, Doaa Mohamed, Antje Beyer, Shunji Yamada, Shaaban A. Mousa, Michael Schäfer. Pro- versus Antinociceptive Nongenomic Effects of Neuronal Mineralocorticoid versus Glucocorticoid Receptors during Rat Hind Paw Inflammation. Anesthesiology. 2018 Apr; 128(4):796-809.

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Affidavit

I, Xiongjuan Li, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic **[Genomic and non-genomic effects of mineralocorticoid receptors and glucocorticoid receptors and their roles of pain modulation]** I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, such as, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Date: 16.02.2018 Xiongjuan Li

Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

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