The relaxin peptide family – potential future hope for neuroprotective therapy? A short review

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Abstract

Since its discovery in the 1920’s the relaxin peptide hormone family has not only grown in number to now seven members (relaxin-1, relaxin-2, relaxin-3, insulin-like peptide (INSL) 3, INSL4, INSL5 and INSL6), but ever more effects, such as vasodilatory, angiogenic, anti-apoptotic, anti-fibrotic and anti-inflammatory, have been linked to them. While relaxin-2 has mainly been investigated in the context of cardiac protection, most comprehensively in the RELAX-AHF and RELAX AHF2 studies, a small number of studies have furthermore assessed the potential neuroprotective effects of especially relaxin-2 and other members of the relaxin family. In this short review we summarise and discuss recent efforts to utilize relaxin hormones for neuroprotection and point out potential future fields of research and translational applications. While many questions still need to be answered, the promising results of the available studies definitely warrant future well-designed studies on neuroprotection by relaxin peptides.

Key Words: relaxin; relaxin-2; neuroprotection; stroke; hemorrhagic shock; cerebral

Introduction

After its discovery in the 1920s, the peptide hormone relaxin was assumed to be a primarily reproductive hormone. Present in all mammals, relaxin mediates the necessary cardiovascular adaptations during pregnancy, such as increased renal blood flow and systemic vasodilation (Wilkinson et al., 2005; Callander and Bathgate, 2010). During the decades since relaxin’s discovery, though, the relaxin family peptides have proven to be more complex than initially suspected. With the identification of the relaxin-3 peptide in 2002, humans are now known to possess seven peptides belonging to the relaxin family (relaxin-1, relaxin-2, relaxin-3, insulin-like peptide (INSL) 3, INSL4, INSL5 and INSL6) (Rosengren et al., 2006; Callander and Bathgate, 2010). Most of the circulating relaxin in the human body, relaxin-2, is expressed by the RLN2 gene. Currently, vasodilatory, angiogenic, anti-apoptotic, anti-fibrotic and anti-inflammatory effects can be linked to relaxin, both in males and females (Sarwar et al., 2017). Relaxin acts through a number of signal transduction pathways, for example via generation of cyclic adenosine monophosphate (cAMP), nitric oxide (NO), cyclic guanosine monophosphate (cGMP), or activation of mitogen-associated protein kinases (MAPKs). Its vasodilatory effects can especially be traced back to interaction with the nitric oxide system, and all of the three subtypes (endothelial, inducible and neuronal) of NO synthases (NOS) (Sarwar et al., 2017). The varied effects of relaxin-2 suggest differing relaxin receptor densities which might be the cause for region-specific control of various signal transduction pathways. RXFP1 and RXFP2 represent the known relaxin-2-activated receptors (Bathgate et al., 2013). These receptors are involved in various aspects of the remodeling of cerebral parenchymal arterioles (Chan and Cipolla, 2011; Chan et al., 2013), as well as in the reduction of vascular resistance, and in an increased blood flow in renal and systemic small arteries in humans and rats (Debrab et al., 2006; Conrad and rars Shroff, 2011); these effects, however, often require longer timescales. Area-specific distribution of RXFP1-mRNA expression and relaxin binding sites of the rat brain are well documented (Ma et al., 2012). While relaxin-2 has roughly nanomolar affinities for both receptors (Halls et al., 2015), the affinity is about an order of magnitude higher for the RXFP1 receptor.

While the last years have seen major advances in uncovering the relaxin family’s effects and their underlying mechanisms, many basic concepts and especially potential therapeutic benefits remain uncertain (Callander and Bathgate, 2010; Sarwar et al., 2017).

In recent years, relaxin-2’s therapeutic potential has been mainly investigated in the context of cardiovascular diseases. Animal models initially presented promising results of improved healing and protection against necrotic cell death of cardiomyocytes in ischemia-reperfusion injury, as well as partly reduced infarction size in myocardial ischemia models (Sarwar et al., 2017). Relaxin-2’s physiologic properties, which include reduced proliferation of fibroblasts and pro-inflammatory cytokines as well as vasodilation, therefore seem to recommend the peptide hormone as a therapeutic drug in cardiac illnesses (Ghosh et al., 2017). The RELAX-AHF (international multicenter phase III) study, which enrolled 1,161 patients with acute heart failure (AHF), initially reported positive results of reduced 180-day mortality and a reduction of dyspnea within the first 5 days of treatment (Ghosh et al., 2017). The succeeding RELAX-AHF2 study, a global multicenter phase III study with 6,600 patients, was supposed to provide further details on the therapeutic potential of serelaxin, but ultimately failed to meet its endpoints of reducing cardiovascular...
mortality and preventing the worsening of heart failure (No authors listed, 2017).

While relaxin-2's therapeutic effects have primarily been investigated in a cardiac context, the very same properties suggest a potential neuroprotective effect of relaxin-2 as well.

**Current Research on Relaxin in a Neuropathophysiological Context**

Few studies so far have specifically examined relaxin hormones as potential neuroprotective agents. Nonetheless, the few studies that did reported mainly favorable results and the substance's general properties furthermore appear to warrant more in-depth research into its potential neuroprotective properties.

Wilson et al. (2005) investigated the potential neuroprotective effects of relaxin-2 in a rat stroke model. Thirty minutes before a middle cerebral artery occlusion (MCAO), rats received an intracortical injection of relaxin-2. A 2,3,5-triphenyltetrazolium chloride (TTC) stain subsequent to brain removal four hours post-intervention revealed that relaxin-2 pre-treated animals exhibited a significantly reduced infarct size as compared to saline-injected control animals (Wilson et al., 2005). At this point, Wilson et al. already hypothesized that the neuroprotective effect that could be observed in relaxin-2-treated rats might be due to NO-mediated vasodilation and subsequently improved collateral perfusion through nearby vasculature. An alternative explanation considered estrogen receptor activation through relaxin-2 as the cause of the observed neuroprotection, since relaxin-2 was known to activate estrogen receptors in the uterus and preceding studies had demonstrated that estrogen pre-treatment similarly reduced ischemia-related infarct sizes (Wilson et al., 2005).

Following up on these early results and investigating their initial assumptions, Wilson et al. published another study on relaxin-2 in the following year (Wilson et al., 2006). While basically the same study design (MCAO, brain removal after 4 hours and TTC-staining) was employed, a number of new experimental groups were added, namely combined relaxin-2 and estrogen injection, relaxin-2 and estrogen receptor antagonist injection (ICI 182,780), as well as intravenous injection of relaxin-2 as well as relaxin-2 in combination with an endothelial NOS (eNOS) inhibitor (l-NIO) (Wilson et al., 2006). These additional experimental groups revealed that relaxin-2's neuroprotective effects are eNOS related and not dependent on estrogen receptor activation. While combined relaxin-2 and estrogen injection revealed an additive (insignificant) decrease of infarct size, the injection of an estrogen receptor antagonist did not abolish relaxin-2’s effects – unlike eNOS inhibition, which significantly reduced the neuroprotective effect of relaxin-2 (Wilson et al., 2006). Exclusive relaxin injection again demonstrated a reduction of infarct size in the rat stroke model, both through intracortical and intravenous administration (Wilson et al., 2006).

A more recent study by Bergeron et al. (2015) again confirmed relaxin-2’s neuroprotective effect and furthermore explored in a more in-depth way the underlying mechanisms. While also utilizing a rat stroke model, Bergeron et al. (2015) employed both relaxin-2 and relaxin-3 receptor expression through real-time PCR and assessed the animals’ brains for infarct size. Furthermore, the study employed a wide range of experimental groups that varied in the manner of the MCAO intervention (permanent or transient), the time point of relaxin administration (before or after intervention) and the additional injection of receptor antagonists. Both relaxin-2 and relaxin-3 provided neuroprotection during permanent MCAO, regardless of the time of administration. In confirmation with Wilson et al.’s earlier study, eNOS inhibition abolished these effects. Relaxin receptor (Rxfp) densities varied: Rxfp1 expression was significantly higher than Rxfp3 expression in the cortex, while astrocytes revealed higher Rxfp3 expression (Ma et al., 2006; Bergeron et al., 2015). Interestingly, relaxin-2 could not reduce infarct volume during transient MCAO, while relaxin-3 was again successful in reducing infarct size as compared to saline-treated control animals (Bergeron et al., 2015). As an explanation, Bergeron et al. (2015) refer to the promiscuous binding of relaxin-3 to both Rxfp1 and Rxfp3, while relaxin-2 only activates Rxfp1 (and Rxfp2, albeit this was not subject of this study). The authors therefore propose, that Rxfp1’s vasodilatory effects (triggered by both relaxin-2 and relaxin-3) are responsible for the neuroprotective effects during permanent MCAO, while the neuroprotective effects of relaxin-3 during the transient MCAO (basically an ischemia-reperfusion injury) is based on protection from apoptotic mechanisms mediated by Rxfp3 on a cellular level (Bergeron et al., 2015).

Finally, relaxin-2 has furthermore already been once (to our knowledge) tested on human subjects who had suffered a stroke. Milia et al. (2013) describe a trial performed in a rehabilitation unit in Italy in 2013, consisting of 18 patients who received relaxin-2 and rehabilitation as compared to 18 patients who were treated with rehabilitation alone. All participants had suffered from an ischemic stroke in the previous three weeks. The Modified Rankin Scale (mRs), the Trail Making Test (TMT) and Functional Independence Measure (FIM) were employed to compare patients and to assess potential progress. The TMT and mRs exhibited better scores for relaxin-treated patients at both temporal evaluation points (after 20, respectively 40 days), while the FIM revealed no significant differences at day 20 but a clear benefit on day 40 (FIM 96 as compared to non-relaxin-treated patient group with 75). The authors note that no obvious adverse effects were noticed during relaxin treatment (Milia et al., 2013). As stated in the paper, relaxin-2 was administered in a dose of 40 µg per day (per patient), supposedly orally. Compared to other studies, e.g., RELAX-AHF (Ghosh et al., 2017), this dose seems to be extremely low (roughly 50–100 times lower). Furthermore, data on plasma concentrations obtained by this dosage were not presented, which raises the question as to whether the used amount of relaxin-2 could
activate the relaxin-receptors at all (Table 1).

Future Neuroprotective Application of Relaxin?

While the results of the studies discussed above justify an optimistic outlook on the potential of relaxin peptide hormones as neuroprotective agents, it has to be clearly stated that our current knowledge on the exact mechanisms through which they exert their effects is incomplete, and that further in-depth research is required. While all three of the rat experimental models reported a reduced infarct size in relaxin-treated rats, their results were only based on staining procedures performed after brain removal. To elucidate both the safety and the effectiveness of relaxin treatment, more studies are needed. A wider array of brain specific parameters and longer observation periods of the experimental animals should be included into future more comprehensive efforts. Noteworthy is that none of the studies documented vital parameters (Wilson et al., 2005, 2006; Bergeron et al., 2015) – this even includes Milia et al.’s study on humans, that, while stating that no adverse effects were observed, similarly did not report basic vital parameters (Milia et al., 2013). An apparent difference regarding the baseline values of the TMT and mRS scores of relaxin-treated patients and the control group is likely due to the randomization procedure of the small patient cohort that was included (Milia et al., 2013).

Bischoff et al. (2016) have performed a study on the microcirculatory effects of serelaxin (human recombinant relaxin-2) in a sheep model. After injection of 30µg/kg serelaxin, an increased cortical cerebral blood flow (CBF) was observed through Laser Doppler flowmetry and sidestream dark-field imaging, but not a concomitant increase in subcortical CBF. Expression levels of Rxfp1 and Rxfp2 were additionally investigated and interestingly did not differ between cortex and subcortex, which might indicate that the greater cortical CBF response is due to area-specific differences in signal transduction pathways (Bischoff et al., 2016). This study’s results therefore correspond to the vasodilation and improved circulation described by Wilson et al. (2005, 2006) and Bergeron et al. (2015). These effects could conceivably be utilized in situations of cerebral hypoperfusion. Severe hemorrhagic shock is known to transcend the limits of the cerebral autoregulation and to lead to concomitant cerebral damages through hypoperfusion (Rickards, 2015). Cortical regions are more prone to damages during these situations than the subcortex (Heckbert et al., 1998), the reasons for these disparate occurrences of cerebral damages are currently not known. A systematic review by Nistor et al. demonstrated that, while some promising approaches are currently under research, to date no specific neuroprotective strategies are available that would offer specific protection from cerebral damages due to hemorrhagic shock (Nistor et al., 2017). The context of cerebral damages due to states of hemorrhagic shock might therefore represent a further field of the potential application of relaxin-2 as a neuroprotective agent – especially since the hormone’s NO-mediated vasodilation might mitigate cellular damages. The relatively new discovered relaxin-3 might represent an interesting new field of research as well, both in the context of an elementary understanding of neurophysiology and its potential thera-

| Table 1 General overview of included studies |
|---------------------------------------------|
| Model | Intervention | Total number of animals/human | Extent of stroke | Observation period of animals after intervention (none = direct euthanasia) | Detected Vital Parameters (e.g., Blood Gases, Hemodynamics, Blood Pressure, Heart Rate, Temperature) | Detected Brain Specific Parameters (e.g., ICP, CPP, CBF, rScO2) | Detected brain specific histopathological treatments (infarct size, neuronal cell damage) | Detected long term results, cognitive function | Other specific parameters in brain tissues |
|-------|--------------|-------------------------------|-----------------|-------------------------------------------------|-----------------------------------------------------------------|---------------------------------|-------------------------------------------|------------------------------------------|------------------------------------------|
| Bergeron et al. (2015) | Rats | MCAO normal saline vs. bolus H2 or H3 and H2 or H3 plus L-NIO | 110 | Permanent and transient | --- | --- | --- | --- | --- |
| Milia et al. (2013) | Human | consecutive post stroke patients | 32 | n.s. | --- | --- | --- | --- | --- |
| Wilson et al. (2006) | Rats | MCAO normal saline vs. bolus H2 and 17β-estradiol; or H2 and ICI | 49 | Permanent | 4 h; None | --- | --- | --- | --- |
| Wilson et al. (2005) | Rats | MCAO normal saline vs. bolus H2 | 14 | Permanent | 4 h; None | --- | --- | --- | --- |

CBF: Cerebral blood flow; CPP: cerebral perfusion pressure; ICP: intracranial pressure; rScO2: regional cerebral oxygen saturation; n.s.: not specified; ICI: ICI 182,780, 7α-[9-[(4,4,5,5,5-pentafluoropentyl)sulfanyl]nonyl]-estra-1,3,5(10)-triene-3,17β-diol; H2: human recombinant relaxin-2; H3: human recombinant relaxin-3; L-NIO: non-selective inhibitor of all NOS isoforms; OGD: oxygen glucose deprivation; mRS: modified Rankin Skala; FIM: Functional Independent Measure; TMT: Trail Making Test; TTC: 2,3,5-triphenoltetrazolium chloride; h: hours.
Bischoff SJ, Schmidt M, Lehmann T, Irintchev A, Schubert H, Jung Bergeron LH, Willcox JM, Alibhai FJ, Connell BJ, Saleh TM, Wilson Bathgate RA, Halls ML, van der Westhuizen ET, Callander GE, Kocan Open peer review report: new creations are licensed under identical terms.

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Acknowledgments: We are grateful to Prof. Georg Matziolis (Department for Orthopaedics, Rudolf Elle Hospital Eisenberg, Germany) and Prof. Otmar Huber (Institute for Biochemistry II, University Hospital Jena, Germany) for their generous encouragement and support.

Author contributions: MN and RS designed and performed the study, analysed data. MN, MS and RS discussed data, interpreted results, drafted, edited and finally approved the manuscript.

Conflicts of interest: None declared.

Peer review: Externally peer reviewed.

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Open peer review report: Reviewer: Andrew L. Gundlach, The University of Melbourne, Australia.

Comments to authors: A ‘strength’ of the article is the enticing concept that relaxin systems might be neuroprotective.

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