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Hypothesis

Relaxin receptors and nitric oxide synthases: search for the missing link

Silvia Nistri and Daniele Bani*

Address: Dept. Anatomy, Histology & Forensic Medicine, University of Florence. V.le G.Pieraccini, 6 I-50139 Florence, Italy

Email: Silvia Nistri - silvianistri@virgilio.it; Daniele Bani* - daniele.bani@unifi.it

* Corresponding author

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Researchers involved in the study of relaxin have welcomed the article by Hsu *et al.* [1], who eventually identified the receptors for this hormone. In the past, using labeled relaxin, specific binding sites have been found in several target organs and tissues for this hormone [2–4], but the exact molecular and functional nature of the relaxin receptor remained elusive. Relaxin is structurally similar to insulin and insulin-like growth factors (IGFs) [5], hence it seemed logical to assume that its putative receptor should belong to the insulin family of receptors, which are membrane-associated tyrosine kinases. Nonetheless, studies in this direction have been unfruitful and even misleading, as experienced by Dr Ivell and his team. In a recent study they were at a very short step from a crucial discovery, because they found that the relaxin-induced cAMP accumulation in target cells in *in vitro* culture was prevented by a pharmacologic inhibitor of G protein activation [6]. However, their attention being focused on tyrosine phosphorylation, they did not go deep into this finding. Using a completely different approach, Hsu and coworkers noticed that *knock-out* mice for the relaxin-like factor/Insl3 (also known as Leydig cell relaxin) had an abnormal testis descent phenotype [7,8] which was similar to that of mice with a disruption of a G protein-coupled receptor (GPCR) gene [9]. On these grounds, they screened the human and mouse genome for orphan GPCRs and they could smartly demonstrate that relaxin is a cognate ligand for two leucine-rich repeat-containing GPCRs, LGR7 and LGR8, acting through G_s proteins. Activation of adenylate cyclase by G_s proteins can explain why relaxin induces an elevation of cAMP in target cells and tissues [5]. In particular, in the same article cited above [1], Hsu and coworkers demonstrated that LGR7 and LGR8 are capable of mediating the action of relaxin through a cAMP-dependent pathway distinct from that of insulin and IGF family ligands.

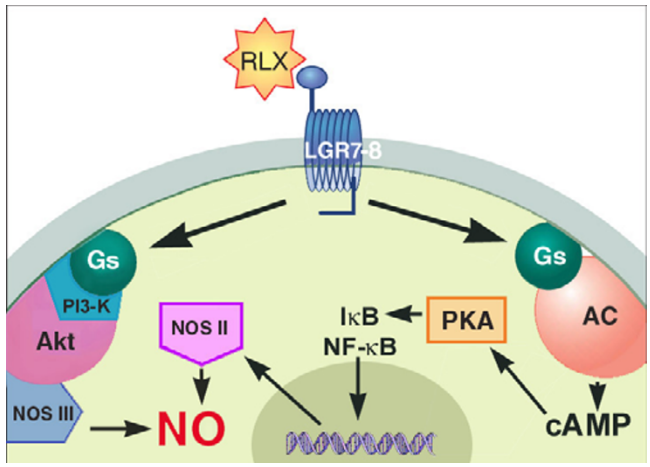

Figure 1

Putative interactions between relaxin receptor signaling and intrinsic NO pathway. AC: adenylate cyclase Akt: protein kinase B Gs: G_s proteins IκB inhibitor subunit of nuclear factor kappa-B LGR7-8: leucine-rich repeat-containing G protein-coupled receptors 7 and 8 NF-κB nuclear factor kappa-B NO: nitric oxide NOS II: inducible NO synthase NOS III: constitutive NO synthase PI3-K: phosphoinositide 3-kinase PKA: protein kinase A RLX: relaxin

In recent years, our own studies and those of other investigators have provided increasing evidence that relaxin can also act on several of its targets by increasing the expression and/or activity of nitric oxide synthase (NOS) isoenzymes, thereby promoting the generation of nitric oxide (NO) [10–16]. Time is ripe for investigating how the newly discovered relaxin GPCRs may account for this. Based on the current literature, there are multiple pathways by

which GPCRs can stimulate NO biosynthesis. In endothelial cells, the best-described agonists for the constitutive NOS III isoform, acetylcholine and bradykinin, activate specific membrane-associated GPCRs [17,18]. Similar effects are exerted by surface estrogen receptors [19]. It has been reported that NOS III can be also activated by direct stimulation of GPCRs with sphingosine 1-phosphate [20]. The classical signaling pathway appears to involve G protein βγ subunits that, by means of phosphoinositide 3-kinase (PI3-K), switch on protein kinase B (Akt), which in turn activates eNOS by phosphorylation at Ser-1179 [20]. Another mechanism, which may either coexist in the same cell or be alternatively operating in different cell types, could involve cAMP and the inducible NOS II isoform. In rat vascular smooth muscle cells, GPCR-activated adenylate cyclase and the consequent rise in cAMP upregulates protein kinase A (PKA) activity [21]. In turn, PKA is able to phosphorilate and inactivate IkB-α, the inhibitor subunit of the transcription factor NF-κB, thus allowing NF-κB to translocate into the nucleus and to promote the expression of NOS II [22]. This latter mechanism may be operating in some relaxin targets, in which induction of NOS II expression and/or high-output, sustained NO generation have been observed [11,12,15,16], NOS II being far more active than NOS III [23]. As summarized in Figure 1, at present there are some clues but no definitive data about the molecular mechanisms by which relaxin, by binding to its GPCRs, may induce the activation of the NO pathway in its targets. Hopefully, future research would clarify this point.

References

1. Hsu SY, Nakabayashi K, Nishi S, Kumagai J, Kudo M, Sherwood OD and Hsueh AJW **Activation of orphan receptors by the hormone relaxin.** *Science* 2002, **295:**671-673
2. Osheroff PL and Ho W **Expression of relaxin mRNA and relaxin receptors in postnatal and adult rat brain and hearts.** *J Biol Chem* 1993, **268:**15193-15199
3. Min G and Sherwood OD **Identification of specific relaxin-binding cells in the cervix, mammary glands, nipples, small intestine, and skin of pregnant pigs.** *Biol Reprod* 1996, **55:**1243-1252
4. Kohsaka T, Min G, Lukas G, Trupin S, Trupin Campbell E and Sherwood OD **Identification of specific relaxin-binding cells in the human female.** *Biol Reprod* 1998, **59:**991-999
5. Sherwood OD **Relaxin.** *in The Physiology of Reproduction (Edited by: Knobil E, Neill JD) New York, Raven* 1994, **1:**861-1009
6. Bartsch O, Bartlick B and Ivell R **Relaxin signalling links tyrosine phosphorylation to phosphodiesterase and adenylyl cyclase activity.** *Mol Hum Reprod* 2001, **7:**799-809
7. Zimmermann S, Steding G, Emmen JM, Brinkmann AO, Nayernia K, Holstein AF, Engel W and Adham IM **Targeted disruption of the Insl3 gene causes bilateral cryptorchidism.** *Mol Endocrinol* 1999, **13:**681-691
8. Nef S and Parada LF **Cryptorchidism in mice mutant for Insl3.** *Nat Genet* 1999, **22:**295-299
9. Overbeek PA, Gorlov IP, Sutherland RW, Houston JB, Harrison WR, Boettger-Tong HL, Bishop CE and Agoulnik AI **A transgenic insertion causing cryptorchidism in mice.** *Genesis* 2001, **30:**26-35
10. Bani D **Relaxin: a pleiotropic hormone.** *Gen Pharmacol* 1997, **28:**13-22
11. Masini E, Bani D, Bello MG, Bigazzi M, Mannaioni PF and Bani Sacchi T **Relaxin counteracts myocardial damage induced by ischemia-reperfusion in isolated guinea pig hearts: evidence for an involvement of nitric oxide.** *Endocrinology* 1997, **138:**4713-4720
12. Bani D, Failli P, Bello MG, Thiemermann C, Bani Sacchi T, Bigazzi M and Masini E **Relaxin activates the L-arginine-nitric oxide pathway in vascular smooth muscle cells in culture.** *Hypertension* 1998, **31:**1240-1247
13. Bani D, Baccari MC, Nistri S, Calamai F, Bigazzi M and Bani Sacchi T **Relaxin upregulates the nitric oxide biosynthetic pathway in the mouse uterus. Involvement in the inhibition of myometrial contractility.** *Endocrinology* 1999, **140:**4434-4441
14. Danielson LA, Sherwood OD and Conrad KP **Relaxin is a potent renal vasodilator in conscious rats.** *J Clin Invest* 1999, **103:**525-533
15. Failli P, Nistri S, Quattrone S, Mazzetti L, Bigazzi M, Bani Sacchi T and Bani D **Relaxin up regulates inducible nitric oxide synthase expression and nitric oxide generation in rat coronary endothelial cells.** *FASEB J Dec. 14, 2001, 10.1096/fj.01-0569fje. Summary* 2002, **16:**252-254
16. Bani D, Baccari MC, Quattrone S, Nistri S, Calamai F, Bigazzi M and Bani Sacchi T **Relaxin depresses small bowel motility through a nitric oxide-mediated mechanism. Studies in mice.** *Biol Reprod* 2002, **66:**778-784
17. Liu J, Conklin BR, Blin N, Yun J and Weiss J **Identification of a receptor/G-protein contact site critical for signaling specificity and G-protein activation.** *Proc Natl Acad Sci USA* 1995, **92:**11642-11646
18. Vanhoutte PM **Endothelial dysfunction and atherosclerosis.** *Eur Heart J* 1997, **18:**E19-E29
19. Wyckoff MH, Chambliss KL, Mineo C, Yuhanna IS, Mendelsohn ME, Mumby SM and Shaul PW **Plasma membrane estrogen receptors are coupled to endothelial nitric oxide synthase through $G\alpha_i$.** *J Biol Chem* 2001, **276:**27071-27076
20. Igarashi J and Michel T **Sphingosine 1-phosphate and isoform-specific activation of phosphoinositide 3-kinase beta. Evidence for divergence and convergence of receptor-regulated endothelial nitric-oxide synthase signaling pathways.** *J Biol Chem* 2001, **276:**36281-36288
21. Kim NY, Pae HO, Kim YC, Choi CK, Rim JS, Lee HS, Kim YM and Chung HT **Pentoxifylline potentiates nitric oxide production in interleukin-1beta-stimulated vascular smooth muscle cells through cyclic AMP-dependent protein kinase A pathway.** *Gen Pharmacol* 2000, **35:**205-211
22. Grimm S and Baeuerle PA **Review article: the inducible transcription factor NF-κB: structure-function relationship of its protein subunits.** *Biochem J* 1993, **290:**297-308
23. Moncada S, Palmer RMJ and Higgs EA **Nitric oxide physiology, pathophysiology, and pharmacology.** *Pharmacol Rev* 1991, **43:**109-142