Proceedings of the Estonian Academy of Sciences,
2024, **73**, 3, 193–196
https://doi.org/10.3176/proc.2024.3.03
Available online at www.eap.ee/proceedings

BIOCHEMISTRY

Beyond the discovered peptides by Viktor Mutt – a contribution[1]

Ülo Langel[a,b]

[a] Department of Biochemistry and Biophysics, Stockholm University, Stockholm SE-10691, Sweden; ulo.langel@dbb.su.se
[b] University of Tartu, Ülikooli 18, 50090 Tartu, Estonia

Received 2 April 2024, accepted 22 April 2024, available online 21 June 2024

Abstract. A summary of the results obtained in the collaboration between the laboratory of Prof. Viktor Mutt at Karolinska Institutet and our laboratory at Stockholm University, headed by Prof. Tamas Bartfai, is given. It is demonstrated that the contribution of Viktor Mutt to the discovery and study of bioactive peptides has led to several further developments, which extend far beyond the original goals of his study and allow us to discuss their importance today and in the future. This perspective has made the contribution of Viktor Mutt to bioactive peptide research excellent and extraordinary.

Keywords: peptides, cell-penetrating peptides, chimeric.

INTRODUCTION

In 1987, when I joined the laboratory of Professor Tamas Bartfai at Stockholm University, I was introduced to Viktor Mutt, who was a professor of biochemistry at Karolinska Institutet, as our labs had several common interests in galanin research. It was the time when scientists had to go to a good library to read journals and copy scientific articles. As Karolinska Institutet had such a library, we often visited this place, and, in most cases, I took the liberty to visit Viktor in his office, where we had discussions on interesting scientific topics. His office was small and full of books and copies of articles. However, if we needed a paper for discussion, he was always able to find this material in the piles of articles, and this was very impressive to all his visitors.

During our common project, I learned a lot from Viktor, and I remember most vividly his commitment to peptide chemistry and pharmacology, which allowed him to make fundamental discoveries in this field. I am still very thankful to Viktor for his opinions and suggestions.

In the title of this paper, the phrase ‘a contribution’ refers to the research that started from Viktor’s discoveries of multiple bioactive peptides and led to further research with several surprising results, far beyond the original goal of the study.

Galanin

Neuropeptide galanin was discovered by Viktor Mutt in 1983 and was immediately recognized for its several important biological activities (Tatemoto et al. 1983). It was of great interest to characterize galanin’s receptors, and this was one of the goals of our cooperation as we were working with CNS receptors in Professor Bartfai’s lab at that time.

We started our galanin research from the regular synthesis of this peptide and its analogues. As the NMR structure of the rat brain galanin was available (Wennerberg et al. 1990), it was possible to obtain information concerning the pharmacophores that are responsible for the receptor recognition. These studies were made together with

[1] This article is based on the lecture presented by Ülo Langel at the conference commemorating the 100th birthday of Viktor Mutt at the Estonian Academy of Sciences, Tallinn, on 19 January 2024.

Tiit Land, and the results were later used for the design of galanin receptor subtype selective ligands and the study of their biological properties. These investigations yielded several interesting results, not directly related to the galanin pharmacophore research (Webling et al. 2012).

Chimeric ligands

Chimeric peptide ligands were made by coupling the sequence from N-terminus of galanin with some other neuropeptides, which were known to have activity at C-terminus (Land et al. 1991). Schematically the structure of such chimeric peptides is shown in Fig. 1.

It was found that some combinations of two neuropeptides were recognized by both neuropeptide receptors, and, most interestingly, there was a significant synergistic effect on binding effectiveness, with the improvement of this parameter by 1000 times. Thereafter, many of these ligands were synthesized and tested with galanin receptor subtypes, which had become available at that time and were expressed in cell lines.

It was suggested that these chimeric ligands may bind to different receptors simultaneously, resulting in a synergistic effect (Langel et al. 1992). Although this paper remained practically unnoticed, as it has had only 100 citations in 30 years, the idea about receptor dimerization has been more widely introduced and recognized many years later. Even more, the concept of receptor multimerization is generally accepted today, and the co-functioning of several receptor mosaics has been demonstrated. Besides combinations of two neuropeptides, some other chimeric ligands were prepared, where galanin was connected with a 'fantasy sequence', or just with single amino acids, such as alanine, leucine, etc. (Bartfai et al. 1993).

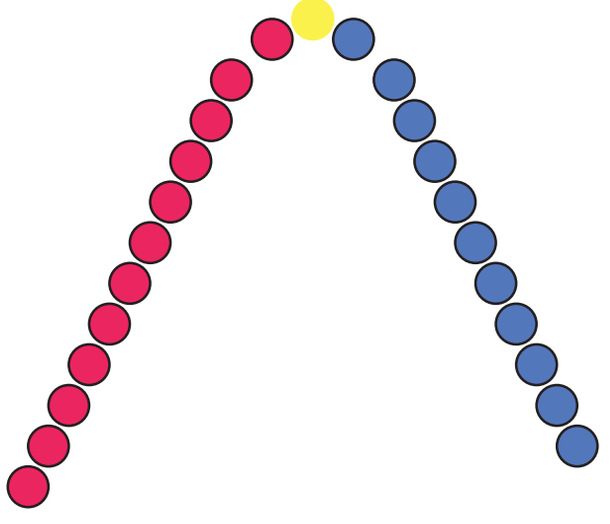

Fig. 1. General principle for designing chimeric ligands for the galanin receptor. In red: sequence from N-terminus of galanin, in blue: sequence from C-terminus of other bioactive peptides, in yellow: amino acid proline causing the bent structure of the obtained peptide.

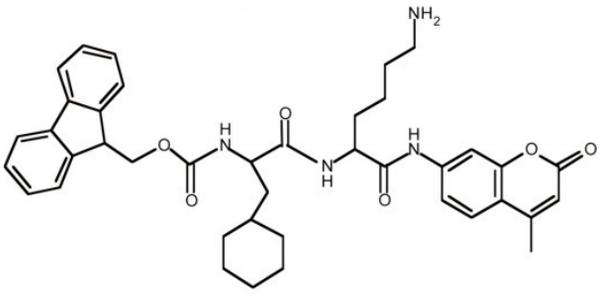

Fig. 2. Low-molecular weight galanin receptor ligand galnon (Saar et al. 2002).

There was a special interest in obtaining non-peptide ligands for the galanin receptor. Several compounds were designed and prepared, such as galnon (Fig. 2).

These small molecules had their effect at micromolar concentrations but revealed very interesting properties. There are several publications about this ligand (Saar et al. 2002) as well as about other synthetic peptides, suggesting the possibility that such substances could have importance in the future. This possibility is confirmed by a recent publication about the ligand M871, which is a selective antagonist of the galanin receptor subtype 2 and seems to have its role in craniofacial development (Palominos et al. 2023).

Drug delivery

Drug delivery has become a very important research field since the size of efficient drug molecules becomes larger and larger, and the use of special delivery vehicles is needed to get these drugs into the cell or across the blood-brain barrier. Among different delivery vectors in use are peptides, known as cell-penetrating peptides (CPP) (Langel 2021, 2023). Transporters proposed by our research group are based on the chimeric galanin-based peptides, which were discussed before (Cerrato et al. 2014). Cargos transported by CPP can be very different, including peptides, proteins, nucleic acids, small synthetic molecules, and even cells.

Basically, the best CPP leads were transportan and transportan10, which contain a part of the galanin sequence, coupled at the C-terminus of mastoparan (Soomets et al. 2000). These CPPs are in wide use, in both in vitro and in vivo studies. For example, in the case of targeted tumour delivery of the drugs, we do not want to kill other

tissues than cancer cells. This is a tricky task as, in vivo, we may get inflammatory and immunogenic responses in other cells if we do not achieve precise targeting of the drugs. Therefore, we concentrated on the development of cell-penetrating peptides to deliver anti-cancer drugs to cancer cells as precisely as possible (Eriste et al. 2013; Veiman et al. 2015).

Hundreds of such delivery peptides have been reported in our laboratory, including PepFects and NickFects (Langel 2023, 2021). These peptides can be used for the delivery of nucleic acids but also other cargoes, attached by using covalent and noncovalent binding strategies. By adding homing sequences, these ligands can be targeted to different tissues. There are examples that have great potential for gene silencing and gene therapy. Our PepFect, coupled with oligonucleotides, forms nanoparticles whose uptake was demonstrated by electron microscopy (El-Andaloussi et al. 2011; Ezzat et al. 2011). Injection of siRNA under in vivo conditions was used to achieve the knockdown of luciferase synthesis. Conjugation of a tumour-targeting peptide with CPP resulted in tumour growth inhibition in some cases (Künnapuu et al. 2019; Veiman et al. 2015). Moreover, in some cases, labelling of glioma cells was observed after the intravenous injection of the targeting peptide called the gHoPe2 peptide (Eriste et al. 2013). Therefore, this research seems to open good perspectives for the design of new drugs, based on specific delivery methods.

CONCLUSION

It is important to emphasize that all these new trends in cell-penetrating-peptide chemistry and the modern design of targeted drugs are based on, or rather directly related to the studies of biologically active neuropeptides, discovered by Viktor Mutt almost four decades ago.

ACKNOWLEDGEMENT

The publication costs of this article were covered by the Estonian Academy of Sciences.

Photo: Estonian Academy of Sciences/Reti Kokk

Ülo Langel is Professor Emeritus at the Department of Biochemistry and Biophysics, Stockholm University, and at the Institute of Technology, University of Tartu. His professional experience includes a career at the University of Tartu, Tartu, Estonia (from Junior Research Fellow to Associate Professor, Visiting Professor and Professor, 1974–present); The Scripps Research Institute, La Jolla, CA, USA (Associate Professor and Adjunct Professor, 2000–present); and Stockholm University, Stockholm, Sweden (from Research Fellow to Associate Professor, Professor and Chairman, 1987–present). He is an Honorary Professor at the University of Ljubljana, Ljubljana, Slovenia. He was Chairman of the Board of small companies Pepfex (Sweden) and Cepep (Estonia) and a member of the Board of Orexo (Sweden). In 2013, he was elected a member of the Academia Europaea and in 2015, a foreign member of the Estonian Academy of Sciences. He has been awarded the Order of the White Star, 4th class, by the Republic of Estonia. He has been an invited lecturer at numerous international conferences and is a coauthor of more than 480 scientific articles and 20 approved or pending patents. His research interest is in neurochemistry of neuropeptide receptors and in drug delivery by cell-penetrating peptides.

REFERENCES

Andaloussi, S. E., Lehto, T., Mäger, I., Rosenthal-Aizman, K., Opera, I. I., Simonson, O. E. et al. 2011. Design of a peptide-based vector, PepFect6, for efficient delivery of siRNA in cell culture and systemically in vivo. *Nucleic Acids Res.*, **39**(9), 3972–3987. https://doi.org/10.1093/nar/gkq1299

Bartfai, T., Langel, Ü., Bedecs, K., Andell, S., Land, T., Gregersen, S. et al. 1993. Galanin-receptor ligand M40 peptide distinguishes between putative galanin-receptor subtypes. *Proc. Natl. Acad. Sci. U. S. A.*, **90**(23), 11287–11291. https://doi.org/10.1073/pnas.90.23.11287

Cerrato, C. P., Lehto, T. and Langel, Ü. 2014. Peptide-based vectors: recent developments. *Biomol. Concepts*, **5**(6), 479–488. https://doi.org/10.1515/bmc-2014-0024

Eriste, E., Kurrikoff, K., Suhorutšenko, J., Oskolkov, N., Copolovici, D. M., Jones, S. et al. 2013. Peptide-based glioma-targeted drug delivery vector gHoPe2. *Bioconjug. Chem.*, **24**(3), 305–313. https://doi.org/10.1021/bc300370w

Ezzat, K., Andaloussi, S. E., Zaghloul, E. M., Lehto, T., Lindberg, S., Moreno, P. M. D. et al. 2011. PepFect 14, a novel cell-penetrating peptide for oligonucleotide delivery in solution and as solid formulation. *Nucleic Acids Res.*, **39**(12), 5284–5298. https://doi.org/10.1093/nar/gkr072

Künnapuu, K., Veiman, K.-L., Porosk, L., Rammul, E., Kiisholts, K., Langel, Ü. et al. 2019. Tumor gene therapy by systemic delivery of plasmid DNA with cell-penetrating pep-

tides. *FASEB Bioadv.*, **1**(2), 105–114. https://doi.org/10.1096/fba.1026

Land, T., Langel, Ü., Löw, M., Berthold, M., Undén, A. and Bartfai, T. 1991. Linear and cyclic N-terminal galanin fragments and analogs as ligands at the hypothalamic galanin receptor. *Int. J. Pept. Protein Res.*, **38**(3), 267–272. https://doi.org/10.1111/j.1399-3011.1991.tb01438.x

Langel, Ü. 2021. Cell-penetrating peptides and transportan. *Pharmaceutics,* **13**(7), 987. https://doi.org/10.3390/pharmaceutics13070987

Langel, Ü. 2023. Nucleic acid delivery by cell-penetrating peptides. *Proc. Estonian Acad. Sci.*, **72**(4), 361–370. https://doi.org/10.3176/proc.2023.4.01

Langel, Ü., Land, T. and Bartfai, T. 1992. Design of chimeric peptide ligands to galanin receptors and substance P receptors. *Int. J. Pept. Protein Res.*, **39**(6), 516–522. https://doi.org/10.1111/j.1399-3011.1992.tb00282.x

Palominos, M. F., Muhl, V., Richards, E. J., Miller, C. T. and Martin, C. H. 2023. Jaw size variation is associated with a novel craniofacial function for galanin receptor 2 in an adaptive radiation of pupfishes. *Proc. Biol. Sci.*, **290**(2009), 20231686. https://doi.org/10.1098/rspb.2023.1686

Saar, K., Mazarati, A. M., Mahlapuu, R., Hallnemo, G., Soomets, U., Kilk, K. et al. 2002. Anticonvulsant activity of a nonpeptide galanin receptor agonist. *Proc. Natl. Acad. Sci. U. S. A.*, **99**(10), 7136–7141.

Soomets, U., Lindgren, M., Gallet, X., Hällbrink, M., Elmquist, A., Balaspiri, L. et al. 2000. Deletion analogues of transportan. *Biochim. Biophys. Acta*, **1467**(1), 165–176. https://doi.org/10.1016/s0005-2736(00)00216-9

Tatemoto, K., Rökaeus, A., Jörnvall, H., McDonald, T. J. and Mutt, V. 1983. Galanin – a novel biologically active peptide from porcine intestine. *FEBS Lett.*, **164**, 124–128. https://doi.org/10.1016/0014-5793(83)80033-7

Veiman, K.-L., Künnapuu, K., Lehto, T., Kiisholts, K., Pärn, K., Langel, Ü. et al. 2015. PEG shielded MMP sensitive CPPs for efficient and tumor specific gene delivery in vivo. *J. Controll. Release*, **209**, 238–247. https://doi.org/10.1016/j.jconrel.2015.04.038

Webling, K. E. B., Runesson, J., Bartfai, T. and Langel, Ü. 2012. Galanin receptors and ligands. *Front. Endocrinol.*, **3**, 146. https://doi.org/10.3389/fendo.2012.00146

Wennerberg, A. B., Cooke, R. M., Carlquist, M., Rigler, R. and Campbell, I. D. 1990. A ^{1}H NMR study of the solution conformation of the neuropeptide galanin. *Biochem. Biophys. Res. Commun.*, **166**(3), 1102–1109. https://doi.org/10.1016/0006-291x(90)90980-2

Viktor Muti avastatud bioaktiivsete peptiidide panus tulevikku

Ülo Langel

Artiklis esitatakse ülevaade professor Viktor Muti labori (Karolinska Instituut) ja professor Tamas Bartfai labori (Stockholmi Ülikool) koostöö tulemustest ja nende võimalikust edaspidisest tähtsusest. Viktor Mutt andis väljapaistva panuse bioaktiivsete peptiidide avastamisse ja uurimisse ning kirjeldatud koostöö tulemusena iseloomustati galaniini retseptori alatüüpe ja loodi neile mitmeid selektiivseid ligandeid. Paljud arendused on seotud kimäärsete peptiidligandite loomise ja uurimisega. Samuti on galaniini ja selle analoogide kasutamisel loodud rakku tungivad transportpeptiidid, mis pakuvad huvi ravimite sihitud kohaletoimetamise seisukohast ja ulatuvad kaugemale bioaktiivsete peptiidide uuringute algeesmärkidest, tehes seega Muti panusest erakordse teadussaavutuse.