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NEURO-
PHARMACOLOGY

Neuropeptides and pharmacology[1]

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Abstract. The aim of the article is to briefly describe our work together with Dr. Viktor Mutt. He discovered and purified many new gastrointestinal bioactive neuropeptides that have important applications as therapeutic agents in diabetes, epilepsy, migraine and weight control. Dr. Mutt's strategy was to search for C-terminally amidated peptides. We determined neuropeptide Y equilibrium binding for its receptor. Our work also included the L-Ala scan of pharmacophores of the neuropeptide galanin (1-28). Finally, the work led to the establishment of the coexistence of peptides with small molecule neurotransmitters, such as serotonin, acetylcholine, noradrenaline and dopamine in central and peripheral neurons.

Keywords: neuropharmacology, neuropeptides, transmitter coexistence.

INTRODUCTION

Viktor Mutt was a pioneering biochemist who identified many gastrointestinal neuropeptides that are bioactive and bind with high affinity to multiple receptors. This research has led to the identification of additional peptides that have important applications as therapeutic agents in diabetes, epilepsy, migraine and weight control, among other indications.

Viktor Mutt and Birgitta Werner lived in a modest apartment, which I (T. B.) often visited. I am grateful for the scientific and personal contacts with Viktor Mutt and his clinician wife Birgitta and with the many colleagues working in Viktor Mutt's laboratory on the projects around the peptides that he/they discovered.

I would like to start by showing this picture from a symposium in Stockholm in June 1985 (Photo 1). In the first row, the fifth from the left is Viktor Mutt.

Viktor Mutt's pioneering techniques were based on solid workable solutions at the time (in the 1960s–1980s, when most discoveries were made at the level of gene products), focusing on proteins and peptides rather than on encoding DNAs, RNAs, or cDNAs.

If you want to identify, purify and sequence peptides, you need a lot of starting material. Viktor worked on a set of peptides, which, at the beginning, were only considered as hormones – their circulating active concentrations were expected to be very low and to act with high affinity at selective receptors. The second thing you need for the identification of these peptides is a very sensitive assay. At the beginning, he had been working on insulin and its release from pancreatic islets and its effects on e.g. blood glucose levels, which was then by far the most sensitive bioassay you could think of.

Viktor Mutt's research strategy

Proteins are relatively long-lived (minutes to hours), but they are not entirely stable. They either disappear in the proteasome after ubiquitination or are chopped up by exo-

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

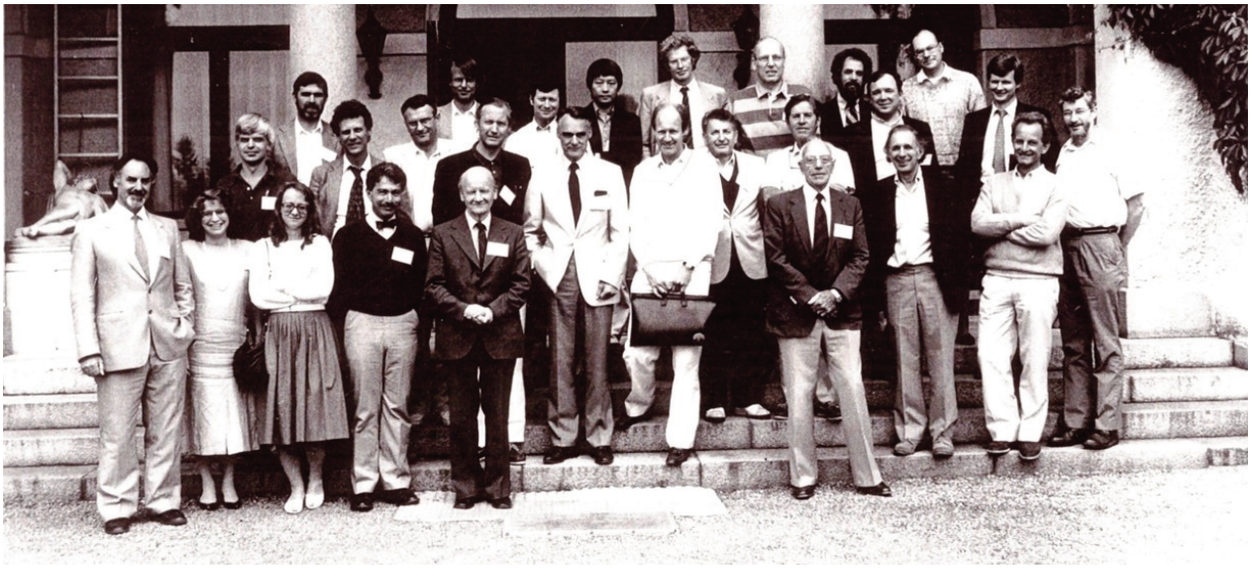

Photo 1. Chairmen and speakers of the Marcus Wallenberg Symposium on Coexistence of Neuronal Messengers: A New Principle in Chemical Transmission, held at the Grand Hotel, Saltsjöbaden, Stockholm, 26–28 June 1985. From the left: Geoffrey Burnstock, Lana Skirboll, Lars Terenius, Marianne Schultzberg, Gösta Jonsson, Larry Swanson, Floyd Bloom, Lennart Stjärne, Lars Olson, Viktor Mutt, Kjell Fuxe, Ira Black, Bengt Pernow, Yuh Nung Jan, Tomas Hökfelt, Tamas Bartfai, Menek Goldstein, Jesse Roth, Anthony Pearse, Sir John Eccles, Michael Brownstein, Jean-Pierre Changeux, Eric Kandel, Rolf Håkanson, Jan Lundberg, Marcello Costa, David Potter. With permission from Elsevier, Progress in Brain Research, 1986, eds T. Hökfelt, K. Fuxe, B. Pernow.

and endopeptidases. There are hundreds and hundreds of peptide fragments around, and these are not C-terminally amidated. If you find a protein or peptide that is C-terminally amidated, it most likely has some signal function. This was a very important insight, which led to Viktor Mutt's work (Tatemoto and Mutt 1978) identifying C-terminally amidated peptides (Table 1).

Then antibodies were generated to peptide fragments from these C-terminally amidated peptides – first polyclonal, and later monoclonal. These antibodies were used but not yet for measuring peptide levels since there was no radioimmunoassay around at that time. Rather, they were used as an immunosorbent to identify peptide family members that had a common antigenic motif with the first identified C-terminally amidated peptide. It was incredibly smart, because then they could use the same extracts to fish with immune adsorption for additional peptides. This is how Viktor discovered peptide families, long before we could clone the cDNAs.

He also tried to purify the receptors. He asked us to synthesize peptides, which, like most hormones, were high-affinity agonists with nanomolar or higher affinity for their receptors, and to biotinylate these peptide agonists. There was no higher affinity binding known than the biotin-avidin binding, and he had tried to isolate receptors this way – solubilizing them and using biotinylated

Table 1. Chemical methods pioneered in Viktor Mutt's lab

Chemical termination of polypeptide hormones, fragmentation analysis and carboxy terminal amidated groups	Tatemoto and Mutt 1978
Chemical method for finding naturally occurring polypeptides	Tatemoto and Mutt 1980
Antibody-recognized motifs to define peptide families	Tatemoto et al. 1982 Sillard et al. 1993
Precursor propeptide or prohormone cDNA studies	Bataille et al. 1982 Gafvelin et al. 1990 Bonetto et al. 1995
Identification of the C-terminally alpha-amidated amino acid in peptides	Schmidt et al. 1987
Chemical detection of natural peptides by specific structures	Norberg et al. 1991

peptides to affinity purify them. Sometimes he was successful. When cDNA techniques became available, the sequence of every known peptide was used.

Thereafter, the distribution of the peptide was also studied using antibodies raised to the peptide KLH-antigen (keyhole limpet superantigen) and the receptor. Distribution was studied by receptor autoradiography. The most common labelling of the peptide ligand was with ^{125}iodine at its tyrosine residues.

All of these peptide receptors seem to belong to the G-protein-coupled receptors (GPCR) Class B. This means that they have many binding sites, not only for the peptide but also for negative and positive allosteric modulators, and that they often oligomerize (in fact, the first top-selling drug, 1,4-benzodiazepine, also known as Valium, is an allosteric modulator of the GABA-ligated chloride channel).

Sometimes you are lucky. Porcine galanin, which Mutt and Tatemoto purified from the porcine intestine has its C-terminal amidated. If Viktor Mutt had been looking for human galanin, which is not C-terminally amidated, the applied technique would not have identified it, even though it has 80% sequence homology to porcine galanin.

Today, the pharmacologically most important peptides, not only commercially but also endocrinologically and clinically, are the incretins: gut and neuropeptides such as the glucagon-like peptide (GLP-1) and the glucose-dependent insulinotropic polypeptide (GIP) (gastric inhibitory peptide, as Viktor Mutt called it).

The GLP-1 receptor agonists and their analogues used are peptides. For example, semaglutide from Novo Nordisk is a GLP-1 peptide modified in such a way that the half-life is prolonged by small modifications of the primary structure to avoid – or slow – peptidase action while not reducing affinity at the receptors. Positions two and eight are replaced with amino acid analogues, and the peptide is then alkylated with a long fatty acid chain, which increases its half-life from two minutes for GLP-1 to about five weeks for semaglutide in humans.

The latest type 2 diabetes drug that is also a potent drug for weight loss in obesity is based on connecting GLP-1 and GIP analogues. Zepbound, FDA-approved in 2024, activates receptors for GLP-1 and GIP to reduce appetite and food intake. 'Zepbound's effectiveness for chronic weight management (weight reduction and maintenance) in combination with a reduced-calorie diet and increased physical activity was established in two randomized, double-blind, placebo-controlled trials of adults with obesity or overweight with at least one weight-related condition' (FDA).

Neuropeptide Y

It is important to note that if these gut peptides were also present in the brain and could be isolated from the brain using the same C-terminally amidated peptide, they might be important – an assumption that turned out to be right.

One of the first peptides isolated by Mutt and Tatemoto from the brain was neuropeptide Y (NPY). We published with them the first paper on the NPY receptors, having synthesized the 36-amino-acid-long NPY. The ligand we used (^{125}I-iodinated NPY) was produced by Ülo Langel. Anders Undén determined its equilibrium binding as sub-nanomolar affinity (Undén and Bartfai 1984; Undén et al. 1984). We also showed that, in the presence of a guanosine triphosphate (GTP) analogue, the apparent affinity of the agonist for the receptors was reduced. This was the very first indication that this neuropeptide receptor is a GPCR, as it became the rule for most gut and neuropeptide receptors that the majority of their receptors are GPCRs Class B.

Galanin

Galanin 1-28 was the first Mutt-peptide that was 'scanned' in its entirety by L-alanine replacement of each of its amino acids in order to find its pharmacophore (Land et al. 1991). This is now a standard technique for defining amino acids in peptide ligands that participate in receptor binding.

Galanin analogues have been synthesized as small molecules with galanin receptor agonist characteristics, keeping the important pharmacophore, tryptophane position two, but all of them had weak affinity. By weak affinity I mean micromolar; acetylcholine is also binding with micromolar affinity to its receptors. To produce galnon (Saar et al. 2002) and galmic (Bartfai et al. 2004) as micromolar agonists is not so bad, and they were useful in showing potential clinical utility of galanin receptor agonists in lowering seizure activity.

Coexistence

There are plenty of studies of peptide coexistence with serotonin, acetylcholine, noradrenaline and dopamine in central and peripheral neurons (Table 2). General principles of the neuronal action by neurons containing both peptides and classical neurotransmitters were developed using the tools and assays described above. It became clear that at low frequency stimulation these nerves and central neurons release the classical transmitter, while at high frequency stimulation they also release the neuropeptide. There is frequency-dependent change in the chemical composition of the synaptic signal that the synaptic receptors find (Bartfai et al. 1988b).

Table 2. Coexistence/coregulation of neuropeptides isolated by Viktor Mutt with classical neurotransmitters

Transmitters	Comments	References
acetylcholine (ACh) – VIP	Affected by acute and chronic changes in neuronal activity induced by disease or drugs. Peripheral nervous system (PNS)	Lundberg et al. 1980 Lundberg et al. 1982 Hedlund et al. 1983 Halldén et al. 1986
	Central nervous system (CNS)	Abens et al. 1984
5HT – substance P – TRH ACh – galanin	Acute and chronic neurophysiological and pharmacological effects on classical neurotransmitters and the coexisting and coreleased peptide-neurotransmitters. PNS and CNS	Hökfelt et al 1987 Bartfai et al. 1988a, 1988b
noradrenaline – NPY 5HT – substance P and neuromedin ACh – galanin	Affect the presynaptic release of each other and interact in determining the postsynaptic effects, amplitude and duration	Serfozo et al. 1986 Solti et al. 1987 Iverfeldt et al. 1989
5HT – tachykinins	A positive forward in synaptic signalling that can be affected by chronic antidepressant treatment that elevates synaptic 5HT levels – the chronic drug treatment produces effects on the classical neurotransmitter and on the coexisting peptides, which contribute to both efficacy and side effects	Nordström et al. 1987 Iverfeldt et al. 1989, 1990

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My (T. B.) gratitude to Viktor Mutt can be summarized like this: when I was elected fellow by the US AAAS (American Association for the Advancement of Science) in 2004, the text was: ‘You are being honored for distinguished contributions to the field of neuropharmacology, including identification of brain acetylcholine receptors, and for pioneering work on several neuropeptide signalling systems’, including muscarinic receptors. The pioneering part came from Viktor Mutt and Tomas Hökfelt. With my colleagues T. Land, Ü. Langel, P. Alberts, J. Järv, K. Iverfeld, G. Fisone, A. Undén and others in my lab, I am standing on their shoulders, and I thank them for the collaboration. The publication costs of this article were covered by the Estonian Academy of Sciences.

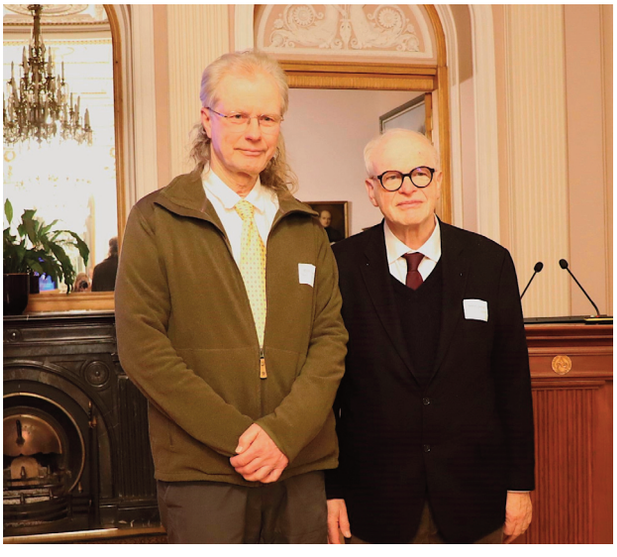

Photo: Estonian Academy of Sciences/Reti Kokk

Pēteris Alberts, PhD, is an Associate Professor at Umeå University, Sweden and an Honorary Doctor of Medicine (Dr. Med. (Honoris Causa)*) of the Latvian Academy of Sciences. He has previously worked as a scientist at the Swedish Defence Research Institute in Umeå, at Pharmacia and Upjohn, and at Biovitrum in Stockholm and Uppsala, Sweden. He also served as a senior scientist at Evolva in Copenhagen, Denmark, and was the Head of Research and Development at Rigvir in Riga, Latvia.*

Tamas Bartfai, PhD, is Professor Emeritus at the University of Stockholm and an Adjunct Professor at the University of Pennsylvania. He has held prominent positions including Vice President for CNS at Hoffmann-La Roche in Basel, Professor and Chair of Molecular and Integrative Neurosciences Department (MIND), The Scripps Research Institute, La Jolla, CA, and Director of Harrold Dorris Neurological Institute, La Jolla, CA, USA. He is a member of several prestigious organizations, including the Swedish Royal Academy of Sciences, Academia Europaea and the Hungarian Academy of Sciences. Additionally, he is a Fellow of the American Association for the Advancement of Science.

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Neuropeptiidid farmakoloogias

Tamas Bartfai ja Pēteris Alberts

Artikkel põhineb loengul, mille Tamas Bartfai pidas dr Viktor Muti 100. sünniaastapäeva puhul Eesti Teaduste Akadeemias. Artiklis kirjeldatakse lühidalt koostööd dr Viktor Mutiga, kes avastas ja puhastas palju uusi seedetraktis esinevaid bioaktiivseid peptiide, mida tänapäeval kasutatakse suurel määral diabeedi-, epilepsia- ja migreeniravis ning ka võitluses ülekaalulisuse vastu. Dr Mutt lõi neuropeptiidide avastamise strateegia, mille kohaselt otsitakse looduslikes objektides C-terminaalselt amideeritud peptiide. Meie kirjeldasime oma uuringute tulemusena neuropeptiidi Y tasakaalulist sidumist selle peptiidi retseptoriga. Lisaks hõlmasid meie uuringud neuropeptiid galaniini (1-28) retseptorite farmakofooride kirjeldamist peptiidijärjestuse L-Ala skaneerimise abil. Uuringute tulemusena iseloomustasime ka neuropeptiidide ja madalmolekulaarsete neurotransmitterite (serotoniin, atsetüülkoliin, noradrenaliin ja dopamiin) koostoimet nii tsentraalsetes kui ka perifeersetes neuronites.