Realia et naturalia

DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS

124

INDREK SAAR

Design of GalR2 subtype specific ligands: their role in depression-like behavior and feeding regulation

DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS
124

DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS

124

INDREK SAAR

Design of GalR2 subtype specific ligands: their role in depression-like behavior and feeding regulation

UNIVERSITY OF TARTU
PRESS

Institute of Chemistry, Faculty of Science and Technology, University of Tartu, Estonia

Dissertation is accepted for the commencement of the degree of Doctor of Philosophy in Chemistry on February 15th, 2013 by the Council of Institute of Chemistry, University of Tartu.

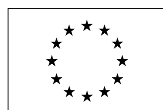
Supervisor: Professor Ülo Langel, PhD,
Institute of Technology, University of Tartu, Estonia;
Chairman, Department of Neurochemistry,
Stockholm University, Sweden

Professor Jaak Järv, PhD, DrSci (Chem),
Institute of Chemistry, University of Tartu, Estonia

Opponent: Professor Barbara Kofler, PhD,
Laura Bassi Centre of Expertise THERAPEP,
Department of Pediatrics/University Hospital Salzburg,
Paracelsus Medical University, Austria

Commencement: Auditorium 1021, Chemicum, 14A Ravila Street, Tartu, at 12.15 on April 5th, 2013

Publication of this dissertation is granted by University of Tartu.



ISSN 1406–0299
ISBN 978–9949–32–235–0 (print)
ISBN 978–9949–32–236–7 (pdf)

Copyright: Indrek Saar, 2013

University of Tartu Press
www.tyk.ee
Order No. 64

ABSTRACT

Galanin is a 29 amino acid long neuropeptide. Through interactions with its three G-protein coupled receptors, GalR1-GalR3, galanin is involved in several functions in the central nervous system, including the regulation of depression-like behavior and food consumption. As all the three receptor subtypes have somewhat different functional binding properties and signaling pathways it has been suggested that they have distinct roles in the regulation of various human diseases and pathological conditions. This makes GalR1-GalR3 unique pharmacological targets. However, due to the lack of subtype specific ligands in the galanin field the involvement of different galanin receptors in various diseases remains to be determined.

This thesis is therefore devoted to the development of novel GalR2 subtype specific ligands. Additionally, these novel compounds and the functions of GalR2 have been investigated in two different behavioral models, namely depression-like behavior and feeding regulation.

Specifically, paper I demonstrates the design of a GalR2 subtype specific ligand and a cell line stably expressing GalR3. In papers II and III more GalR2 subtype specific ligands are introduced and the effect of GalR2 activation on food consumption and depression-like behavior is shown, respectively. Paper IV shows an approach on how to increase the *in vivo* usability of peptide ligands. In addition, it demonstrates a series of systemically active novel GalR ligands with preferential binding to GalR2, including more in-depth characterization of one of these peptide ligands. Moreover, in Paper IV the attenuate effect of these ligands on depression-like behavior is demonstrated.

In conclusion, this thesis provides novel strategies for the design of subtype specific galanin receptor ligands and introduces a series of them, thereby adding additional tools to study the involvement of different galanin receptor subtypes in various disorders. In addition, the involvement of GalR2 and its ligands in depression-like behavior and in the regulation of food consumption has been investigated, and an attenuate effect of several GalR2 agonists on depression-like behavior has been shown.

CONTENTS

LIST OF PUBLICATIONS 9

ADDITIONAL PUBLICATIONS 10

LIST OF ABBREVIATIONS 11

1. INTRODUCTION 13
   1.1. Neuropeptides 13
   1.2. The galanin family 14
      1.2.1. Galanin 15
      1.2.2. Galanin message associated peptide (GMAP) 17
      1.2.3. Galanin-like peptide (GALP) 17
      1.2.4. Alarin 19
   1.3. Galanin receptors 20
      1.3.1. Galanin receptor subtype 1 21
      1.3.2. Galanin receptor subtype 2 21
      1.3.3. Galanin receptor subtype 3 22
   1.4. Biological effects of galanin 22
      1.4.1. Mood disorders and depression 22
      1.4.2. Galanin and depression-like behavior 23
      1.4.3. Energy homeostasis 24
      1.4.4. Galanin and feeding 24
   1.5. Galanin receptor ligands 25
      1.5.1. Peptide ligands 25
      1.5.2. Non-peptide ligands 29

2. AIMS OF THE STUDY 31

3. METHODOLOGICAL CONSIDERATIONS 32
   3.1. Solid phase peptide synthesis 32
   3.2. Galanin receptor ligands (Papers I–IV) 32
   3.3. Radioligand binding assay (Paper I–IV) 33
   3.4. Inositol phosphate accumulation (Paper I–III) 34
   3.5. Depression-like behavior (Paper III–IV) 34
      3.5.1. Tail suspension test 35
      3.5.2. Forced swim test 35

4. RESULTS AND DISCUSSION 36
   4.1. Novel galanin subtype selective ligands (Paper I–IV) 36
   4.2. Galanin in feeding regulation 40
   4.3. Galanin in depression-like behavior 40
   4.4. Development of GalR3 inducible cell line 42

5. CONCLUSIONS AND FUTURE PROSPECTS 43
6. SUMMARY IN ESTONIAN 45
7. ACKNOWLEDGEMENTS 46
8. REFERENCES 47
PUBLICATIONS 59
CURRICULUM VITAE 116

LIST OF PUBLICATIONS

I. Runesson, J., **Saar, I.,** Lundström, L., Järv, J., and Langel, Ü. (2009) A novel GalR2-specific peptide agonist. Neuropeptides, 43, 187–192.

II. **Saar, I.,** Runesson, J., McNamara, I., Järv, J., Robinson, J. K., and Langel, Ü. (2011) Novel galanin receptor subtype specific ligands in feeding regulation. Neurochemistry International, 58, 714–720.

III. **Saar, I.,** Runesson, J., Järv, J., Kurrikoff, K., and Langel, Ü. (2013) Novel galanin receptor subtype specific ligand in depression like behavior. Neurochemical Research, 38, 398–404.

IV. **Saar, I.,** Lahe, J., Langel, K., Runesson, J., Webling, K., Järv, J., Rytkönen, J., Närvanen, A., Kurrikoff. K., and Langel, Ü. Novel systemically active galanin receptor subtype specific ligands in depressioon-like behavior. Journal of Neurochemistry, submitted.

My personal contribution to the articles referred to in this thesis is as follows:

I. synthesized and purified the peptides, performed signaling experiments, participated in the writing of the paper;

II. synthesized and purified the peptides, performed signaling and behavioral experiments, analyzed most of the data, participated in the writing of the paper as corresponding author;

III. synthesized and purified the peptides, performed signaling and behavioral experiments, analyzed most of the data, wrote the paper as corresponding author;

IV. designed all the peptide sequences, participated in the peptide synthesis and purification, analyzed the data, participated in performing behavioral experiments, wrote most of the paper as corresponding author

ADDITIONAL PUBLICATIONS

V. Runesson, J., Groves-Chapman, J. L., Wembling, K., **Saar, I.**, Sillard, R., Holmes, P. V., and Langel, Ü. (2012) Pharmacological stimulation of Galanin receptor 1 but not Galanin receptor 2 attenuates kainic acid-induced neuronal cell death in the rat hippocampus. Neuropeptides, submitted.

VI. Anderson, M. E., Runesson, J., **Saar, J.,** Langel, Ü., Robinson, J. K. Galanin, through the GalR1 but not GalR2 receptors, decreases motivation at times of high appetitive behaviour. Behavioural Brain Research. 239, 90–93.

VII. Morrison, B. E., Marcondes, M. C. G., Nomura, D. K., Sanchez-Alavez M., Sanchez-Gonzalez A., **Saar, I.,** Bartfai, T., Maher, P., Sugama, S., Conti, B. IL-13Rα1 expression in dopaminergic neurons contributes to their loss following chronic systemic treatment with LPS. Journal of Immunology, 189, 5498–5502.

LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine, serotonin
ARC	arcuate nucleus
BBB	blood brain barrier
cAMP	cyclic adenosine monophosphate
cDNA	complementary DNA
CHO	chinese hamster ovary
CNS	central nervous system
CREB	cAMP regulatory element binding protein
CSF	cerebrospinal fluid
DR	dorsal raphe nucleus
Fmoc	9-fluorenylmethoxycarbonyl
FST	forced swim test
GALP	galanin-like peptide
GalR	galanin receptor
GalR1	galanin receptor subtype 1
GalR2	galanin receptor subtype 2
GalR3	galanin receptor subtype 3
GIRK	G protein-coupled inwardly-rectifying potassium channel
GMAP	galanin message associated peptide
GnRH	gonadotrophin-releasing hormone
GRK	G-protein receptor kinases
HPLC	high performance liquid chromatography
i.c.v.	intracerebroventricularly
i.p.	intraperitoneal
IP	inositol phosphate
IP2	inositol 1,4 bisphosphate
IP3	inositol 1,4,5 trisphosphate
Ki	inhibitory dissociation constant
i.t.	intrathecally
KO	knock out
LC	locus coeruleus
LDCV	large dense core vesicles
LH	leutizing hormone
MALDI	matrix-assisted laser desorption/ionization
MAPK	mitogen associated protein kinase
MBHA	p-methylbenzylhydrylamine
mRNA	messenger RNA
NA	noradrenalin
NPY	neuropeptide Y
OE	over expression
PEI	polyethyleneimine
PIP2	phosphatidylinositol 4,5-bisphosphate

PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PNS	peripheral nervous system
PTX	pertussis toxin
PVN	paraventricular nucleus
RT-PCR	reverse transcript polymerase chain reaction
s.c.	subcutaneous
SAR	Structure–activity relationships studies
SPA	scintillation proximity assay
SPPS	solid phase peptide synthesis
SSSE	self-sustaining status epilepticus
SSV	small synaptic vesicles
TFA	trifluoroacetic acid
TIS	triisopropylsilane
TST	tail suspension test
vdW	van der Waals

1. INTRODUCTION

During the last 30 years the pharmaceutical industry has drawn much attention to neuropeptides. Due to the restricted distribution of neuropeptides and their receptors it is assumed that drugs acting on neuropeptide receptors might have less side effects than classical neurotransmitters. Until now, there are several positive examples of drugs for the treatment of chronic diseases targeting G-protein coupled receptors (GPCRs). However, finding selective high affinity non-peptide agonist or antagonist is challenging, and using peptide based compounds as alternatives brings up additional disadvantages, e.g. short half-life as well as a poor ability to cross the blood-brain barrier (BBB).

Since the discovery of galanin in 1983, research in this field has been hampered by the lack of subtype specific ligands. Modern molecular biology and the use of transgenic animals, together with few available ligands, have led the field and helped to delineate the galaninergic system so far. However, the use of transgenic animals often has drawbacks, e.g. tendency of organisms to result in neurochemical compensatory mechanisms when lifelong genetic alterations are being made. Therefore, finding pharmacological tools targeting neuropeptide receptors is of high interest. Recently, the rational drug design for neuropeptide receptors became somewhat easier, as GPCR-ligand co-crystal structures are being published. However, none is yet available for any of the three galanin receptor subtypes.

Research described in this thesis aims to address several of these problems by designing a series of galanin receptor subtype specific ligands (i–iv), and devising an approach to increase *in vivo* usability of ligands by increasing the bioavailability of the peptides in the brain (iv). Furthermore, to test these novel subtype specific ligands in different behavioral settings, and thus aiming to validate galanin receptor subtypes as potential drug targets.

1.1. Neuropeptides

Neuropeptides are 5–50 amino acids-long neuronal signaling molecules with regionally restricted distribution in central and peripheral nervous system. They are transmitter molecules used by nerve cells to communicate with each other and with peripheral cells. They belong to the wide variety of signaling molecules that compose the nervous system.

The first neuropeptide, Substance P, was discovered in 1931 by Euler and Gaddum. However, its sequence was not identified until 40 years later. The identification of neuropeptides accelerated when Victor Mutt and colleagues started focusing on the isolation of C-terminally amidated peptides, thus leading to the discovery of numerous neuropeptides, including galanin, glucagon, secretin, NPY. The field expanded even more following the sequencing of G-protein coupled receptors (GPCRs) by homology cloning. Since many of the

endogenous ligands for the GPCRs were not known, they became a hot drug target for the pharmaceutical industry. This phenomenon was amplified after the Human Genome Project detected numerous GPCR sequences without any knowledge of their endogenous ligands. The majority of neuropeptide receptors belong to GPCRs family, the largest known family of membrane-bound receptors, often also referred to as seven-transmembrane receptors (7TM) which currently constitute one of the favorite classes of drug targets for the pharmaceutical industry.

Neuropeptides exhibit several important differences from classical neurotransmitters. In contrast to neurotransmitters, which are synthesized in nerve terminals, neuropeptides are produced as ribosomal pre-prohormones. These constructs are to be cleaved and often post-translationally modified by amidation, acetylation, phosphorylation, sulfation, and glycosylation. A single neuropeptide precursor molecule can give rise to more than one biologically active peptide, e.g. multiple copies of a single neuropeptide, multiple different neuropeptides or any combination of these. Additionally, the signaling of the low molecular weight neurotransmitters and neuropeptides is somewhat different, since classical neurotransmitters are more widely distributed. Therefore, drugs targeting neuropeptide receptors are hypothesized to be more selective and thus have fewer side effects than the drugs acting on classical neurotransmitter systems. Furthermore, bioactive neuropeptides are released from large dense-core vesicles (LDCV) derived from the trans-Golgi network, whereas the vast majority of classical neurotransmitters are released from small synaptic vesicles (SSV) that are clustered in the synaptic zones. In contrast, LDCVs are more diffusely scattered around the nerve terminals, wherefore the release of neuropeptides from LDCVs generally requires higher stimulation or specific stimulation patterns. SSVs on the other hand can be released by focal increase of calcium in the synaptic zone. Opposite to classical neurotransmitters, no reuptake mechanism is known for neuropeptides, LDCVs can only be used once. Thereafter, the peptides are cleaved and their activity is terminated by extracellular peptidases. On the other hand their postsynaptic effect usually lasts longer than that of the classical neurotransmitters.

Taken together, neuropeptides are molecules that possess a wide spectrum of functions (ranging from neurotransmitter to growth factor), and are of particular importance when the nervous system is challenged. Today, the development of receptor subtype specific ligands for neuropeptide receptors as potential drug candidates or tools to validate GPCRs as drug targets is of high interest.

1.2. The galanin family

Thirty years have passed since the discovery of neuropeptide galanin. During this time three additional bioactive members of the galanin family have been described. Furthermore, three GPCRs that have shown to interact with members of the galanin family have been identified.

1.2.1. Galanin

Galanin was discovered by Professor Viktor Mutt and colleagues using a chemical technique for the detection of amidated peptides at the Karolinska Institute in 1983. The 29 amino acid long neuropeptide was identified from porcine upper intestinal extracts and named galanin after its N-terminal glycine and C-terminal alanine residue (Tatemoto *et al.* 1983). Galanin is C-terminally amidated peptide in most species it has been identified from. Only in humans and macaques is galanin 30 amino acids long and has a C-terminal free carboxylic acid (Table 1).The N-terminal 1–15 amino acids of galanin are conserved in all species, except tuna, where two amino acid residues are substituted (Kakuyama *et al.* 1997). All the differences are situated in the C-terminal region of the peptide. Concordantly, studies have shown that the N-terminal end of galanin is crucial for the biological activity of the peptide, while the C-terminal part is suggested to sterically protect the N-terminal part of the peptide from proteolytic degradation. Comparison of the half-lives of galanin and galanin(1–16) resulted in approximately 3–4 longer time in favor of the entire molecule when measured in membrane suspensions of rat hypothalamus, spinal cord, and in rat cerebrospinal fluid (CSF) (Bedecs *et al.* 1995, Land *et al.* 1991a, Land *et al.* 1991b). In solution, galanin forms a horseshoe-like conformation consisting of two α-helical structures interrupted by a β-bend around the Proline in position 13 (Ohman *et al.* 1998).

Galanin is expressed as a 123 (porcine, bowine and human) and 124 (mouse and rat) amino acid precursor protein called preprogalanin. The propeptide is composed of the N-terminal signal peptide sequence, galanin, and C-terminal 59/60 amino acids long flanking peptide called Galanin Message Associated Peptide (GMAP). Galanin is flanked by tryptic dibasic cleavage sites (Lys-Arg), separating it from its neighbors, the N-terminal signal peptide and GMAP (Rökaeus & Brownstein 1986). As mentioned previously, human and macaque galanin sequences are unique. They differ from all the others due to the single base mutation yielding a Ser in the place of the Gly residue. This substitution results in the C-terminal carboxylic acid, in contrast to galanin from other species, where Gly serves as an amide donor (Cunningham *et al.* 2002, Evans & Shine 1991). The human galanin gene is localized on chromosome 11 at position q13.3–q13.5. The gene spans 6.5 kb and consists of 6 exons which are translated into the 0.9 kb long message (Evans *et al.* 1993).

Galanin is widely expressed in central and peripheral nervous systems as well as in endocrine system of various species, including humans. Its mRNA and galanin-immunoreactivity are distributed throughout the central nervous system (CNS), where it has been mainly identified in hypothalamus, thalamus, ventral hippocampus, locus coerules, midbrain, basal forebrain, medulla oblongata, and in the pituitary (Merchenthaler *et al.* 1993). Furthermore, galanin is known to coexist with other neuropeptides, such as NPY, enkephaline, substance P, vasopressin, calcitonin gene related peptide, and with numerous of classical neurotransmitters like dopamine, serotonin, noradrenaline, ACh, and

GABA (Zhang *et al.* 1998, Zhang *et al.* 1995, Melander *et al.* 1986, Miller *et al.* 1998).

Table 1. Endogenously occurring galanin sequences from different species

Species	Sequence	Reference
Human	GWTLNSAGYLLGPHAVGNHRSFSDKNGLTS	(Schmidt *et al.* 1991)
Macaque	GWTLNSAGYLLGPHAVGNHRSFSDKNGLTS	(Cunningham *et al.* 2002)
Porcine	GWTLNSAGYLLGPHAIDSHRSFHDKYGLA-amide	(Tatemoto *et al.* 1983)
Bovine	GWTLNSAGYLLGPHALDNHRSFQDKHGLA-amide	(Rökaeus *et al.* 1988)
Dog	GWTLNSAGYLLGPHAIDNHRSFHEKPGLT-amide	(Boyle *et al.* 1994)
Sheep	GWTLNSAGYLLGPHAIDNHRSFHDKHGLA-amide	(Sillard *et al.* 1991)
Rat	GWTLNSAGYLLGPHAIDNHRSFSDKHGLT-amide	(Kaplan *et al.* 1988a)
Mouse	GWTLNSAGYLLGPHAIDNHRSFSDKHGLT-amide	(Lundkvist *et al.* 1995)
Chicken	GWTLNSAGYLLGPHAVDNHRSFNDKHGFT-amide	(Norberg *et al.* 1991)
Quail	GWTLNSAGYLLGPHAVDNHRSFNDKHGFT-amide	(Tsutsui *et al.* 1998)
Alligator	GWTLNSAGYLLGPHAIDNHRSFNEKHGIA-amide	(Wang & Conlon 1994b)
Frog	GWTLNSAGYLLGPHAIDNHRSFNDKHGLA-amide	(Chartrel *et al.* 1995)
Tuna	GWTLNAAGYLLGPHGIDGHRTLGDKPGLA-amide	(Habu *et al.* 1994)
Trout	GWTLNSAGYLLGPHGIDGHRTLSDKHGLA-amide	(Anglade *et al.* 1994)
Turtle	GWTLNSAGYLLGPHAVDNHRSLIDLHGLA-amide	(Wang *et al.* 1999)
Bowfin	GWTLNSAGYLLGPHAVDNHRSLNDKHGLA-amide	(Wang & Conlon 1994a)

1.2.2. Galanin message associated peptide (GMAP)

GMAP is the least studied member of the galanin family. The sequence of GMAP shows high degree of homology between species, although not to the same extent as galanin (Table 2) (Lundkvist *et al*. 1995). As expected, the study comparing GMAP- and galanin-like immunoreactivities in the brain demonstrated that the GMAP distribution generally overlaps with that of galanin (Hökfelt *et al.* 1992).

Previous research on several different nociception models has shown that GMAP has pharmacological actions in spinal nociceptive transmission in rat spinal cord (Bartfai *et al.* 1991, Xu *et al.* 1995a, Xu *et al.* 1995b, Andell-Jonsson *et al.* 1997, Hao *et al.* 1999). Recently, interesting studies from Holub and Rauch have demonstrated that GMAP exhibits antifungal activity. A study from 2007 indicates a role of GMAP as a possible new component of the innate immune system. In this publication its inhibitory actions on the growth of *Candida albicans* as well as on the transition of budded-to-hyphal-form has been demonstrated (Rauch *et al.* 2007). In a recent study, Holub *et al.* showed that GMAP was able to reduce the growth of six out of seven *Candida* strains (Holub *et al.* 2011), suggesting therapeutic potential of GMAP as a common antifungal substance.

Table 2. Endogenously occurring GMAP sequences from different species

Species	Sequence	Reference
Human	ELRPEDDMKPGSFDRSIPENNIMRTIIEFLSFHLK EAGALDRLLDLPAAASSEDIERS	(Evans & Shine 1991)
Cow	ELEPEDEARPGSFDRPLAENNVVRTIIEFLTFLH LKDAGALERLPSLPTAESAEDAERS	(Rökaeus & Brownstein 1986)
Rat	ELPLEVEEGRLGSVAVPLPESNIVRTIMEFLSFL HLKEAGALDSLGIPLATSSEDLEQS	(Kaplan *et al.* 1988b)
Mouse	ELQLEVEERRPGSVDVPLPESNIVRTIMEFLSFL HLKEAGALDSLGIPLATSSEDLEKS	(Lundkvist et al. 1995)
Pig	ELEPEDEARPGGFDRLQSEDKAIRTIMEFLAFLH LKEAGALGRLPGLPSAASSEDAGQS	(Lundkvist et al. 1995)

1.2.3. Galanin-like peptide (GALP)

In 1991, the third member of the galanin family, galanin-like peptide (GALP), was isolated from porcine hypothalamus by Ohtaki and colleagues. The 60 amino acid long peptide with non-amidated C-terminus was discovered while searching for endogenous ligands capable to induce GTP-binding to a membrane preparation of GalR2-transfected cells (Ohtaki *et al.* 1999).

The amino acid sequence of GALP (9–21) is identical with the N-terminal 13 residues of galanin, which are known to be conserved in numerous of species and of high importance for the biological activity of galanin. Competitive binding studies with porcine GALP demonstrated its preferential binding (20 times) towards GalR2, when compared to GalR1. In addition, GTPγS binding assay was used to demonstrate porcine GALP agonistic properties (Ohtaki et al. 1999). However, the latest competitive binding studies using human GALP with [^{125}I]-galanin showed that GalR3 binds GALP with the highest affinity (Lang *et al.* 2005).

Analysing the human genome revealed that GALP gene shares a marked degree of similarity with human galanin gene. Both genes are comprised of 6 exons, in which the first is noncoding. Human GALP is located on chromosome 19 (human galanin on chromosome 11) and similarly to preprogalanin, it is encoded by exons 2–6 to a precursor protein. PreproGALP is 115–120 amino acid residues long, varies in different species (Table 3), and is cleaved into GALP peptide (Ohtaki *et al.* 1999, Cunningham *et al*. 2002).

Table 3. Endogenously occurring GALP sequences from different species

Species	Sequence	Reference
Human	APAHRGRGGWTLNSAGYLLGPVLHLPQMGDQ DGKRETALEILDLWKAIDGLPYAHPPQPS	(Ohtaki et al. 1999)
Macaque	APAHQGRGGWTLNSAGYLLGPVLHLPQMGDQ DRKRETALEILDLWKAIDGLPYSHPLQPS	(Cunningham et al. 2002)
Rat	APAHRGRGGWTLNSAGYLLGPVLHLSSKANQ GRKTDSALEILDLWKAIDGLPYSRSPRMT	(Ohtaki et al. 1999)
Mouse	APAHRGRGGWTLNSAGYLLGPVLPVSSKADQG RKRDSALEILDLWKIIDGLPYSHSPRMT	(Juréus *et al.* 2001)
Pig	APVHRGRGGWTLNSAGYLLGPVLHPPSRAEGG GKGKTALGILDLWKIDGLPYPQSQLAS	(Ohtaki et al. 1999)

In contrast to galanin, the distribution of GALP across species is limited. Markable differences in terms of expression patterns/magnitude might be due to the fact that galanin is more widely expressed in the brain than GALP. *In situ* hybridization analysis of rat, mouse, and macaque have revealed cells expressing GALP mRNA to be found in the arcuate nucleus (Arc), median eminence, infundibular stalk, caudal part of the dorsomedial nucleus and in the pituitary (Cunningham et al. 2002, Juréus *et al.* 2000, Takatsu *et al.* 2001). In addition, GALP containing fibers have been predominantly found in hypothalamic paraventricular nucleus, medial preoptic area, the bed nucleus of the striaterminalis, and the lateral septal nucleus (Takatsu *et al.* 2001).

GALP, similarly to galanin, has been shown to be involved in feeding behavior and energy balance. It has short-term orexinergic actions in rats, while in long term it reduces bodyweight and stimulates thermogenesis in rodents. Recent data suggest that long term actions are mediated trough GALP induced expression of Il-1 in the brain. In IL-1α/β-, IL-1β-, or IL-1 type I receptor (IL-1RI)-deficient mice the effects of GALP on feeding, body weight, and body temperature are significantly reduced. Similar effects of GALP were seen when co-administered with IL-1 receptor antagonist (Man & Lawrence 2008). The short-term increased food intake induced by GALP is mediated via orexin and NPY (Kageyama *et al.* 2006, Kuramochi *et al.* 2006).

Several studies have suggested that GALP does not exclusively interact with galanin receptors. It has been shown that similarly to wild type mice, GALP also induces weight loss and food intake reduction in both, GalR1 and GalR2-deficient mice. In addition, GalR2/GalR3 agonist (AR-M1896) does not have any effect on food intake or body weight in rat and mouse (Krasnow *et al.* 2004). All this suggests that there might be an unidentified GALP binding receptor.

1.2.4. Alarin

The most recent member of the galanin family, alarin, is named after its N-terminal alanin and C-terminal serine and was identified in 2006 (Santic *et al.* 2006). The 25 amino acids long neuropeptide is encoded from an alternative transcript of GALP mRNA, which misses exon 3 resulting in a frame shift of the GALP mRNA sequence. It has been shown that Alarin shares very little homology with mature GALP, only the first 5 amino acids are identical. Homology with any other murine peptide, including galanin, has not been detected (Santic *et al.* 2006). Unlike GALP and galanin, alarin lacks the galanin receptor binding domain and thus doesn`t have any binding affinity towards any of the three galanin receptor subtypes (Boughton *et al.* 2010). The sequence identity of alarin between rat and mice is 88%, between human and macaque 96%, while between rodent and primate only 60% (Table 4) (Santic *et al.* 2007). However, similarly to galanin, the N-terminal part of alarin shares homology between all the different species, and therefore, it is suggested that the N-terminal part might be crucial for the receptor binding (Santic *et al*. 2007). This phenomena is somewhat proven by Fraley and colleagues, who demonstrate that removal of several N-terminal amino acids of alarin eliminates its ability to stimulate food consumption and plasma LH secretion (Fraley *et al.* 2012b). However, this study also shows that i.c.v. injected Ala6-25Cys leads to a significant reduction in circulating LH levels and co-administration of it with Ala1-25 prevents the increase in 24 h body weight, suggesting the antagonistic properties of Ala6-25Cys (Fraley et al. 2012b).

Alarin expression has been reported in murine brain, thymus, and skin (Santic *et al*. 2007), human neuroblastic tumors and human skin (Santic et al.

2007, Santic *et al.* 2006). Additionally, a recent study has demonstrated the distribution of alarin-like immunoreactivity (alarin-LI) in rodents' brain more in detail. In CNS of mice, alarin-LI was widely distributed throughout the brain, showing high intensity in the mitral cell layer of the olfactory bulb, the accessory olfactory bulb, the medial preoptic area, different nuclei of the hypothalamus such as the arcuate nucleus and the ventromedial hypothalamic nucleus, the locus coeruleus, the ventral cochlear nucleus, and the facial nucleus and the epithelial layer of the plexus choroideus (Eberhard *et al.* 2012).

Table 4. Endogenously occurring alarin sequences from different species

Species	Sequence	Reference
Human	APAHRSSTFPKWVTKTERGRQPLRS	(Santic et al. 2006)
Rat	APAHRSSTFPQRPTRAGRQTQLLRS	(Santic et al. 2007)
Mouse	APAHRSSPFPPRPTRAGRETQLLRS	(Santic et al. 2007)
Macaque	APAHRSSTFPKWVTKTGRGRQPLRS	(Santic et al. 2007)

Similarly to galanin and GALP, alarin has shown to regulate feeding. Recent research has demonstrated that central administration of alarin increases acute food consumption, while no effect was seen on food intake after 24 h (Fraley *et al.* 2012a, Fraley *et al*. 2012b, Van Der Kolk *et al.* 2010, Boughton *et al*. 2010). Interestingly, the relative bodyweight was shown to be significantly increased after 24 h (Fraley *et al*. 2012a). In addition, i.c.v. injected alarin significantly increases plasma leutizing hormone (LH)-level in a gonadotrophin-releasing hormone (GnRH)-mediated manner (Fraley *et al.* 2012a, Boughton *et al*. 2010, Van Der Kolk *et al.* 2010). Unlike galanin and GALP, alarin has been shown to have no effect on body temperature or on male sex behaviors in rodents (Van Der Kolk *et al.* 2010, Fraley *et al.* 2012a).

1.3. Galanin receptors

The biological activity of galanin is mediated through three galanin receptor subtypes, GalR1, GalR2, and GalR3. All of them are members of G-protein-coupled receptors superfamily and exhibit high binding affinity towards galanin. However, they (GalR1-GalR3) have somewhat different functional binding properties and signaling pathways, which makes` them a great target for the treatment of various human diseases and pathological conditions.

1.3.1. Galanin receptor subtype 1

The first known galanin receptor, GalR1, was cloned from human Bowes melanoma cells (Habert-Ortoli *et al.* 1994). The gene for GalR1 is comprised of 3 exons, which in mouse and human are translated into a 348 and 349 amino acid long proteins, respectively. The homology between species is high, human and mouse GalR1 share 93% identity in the amino acid sequence level (Jacoby *et al.* 1997). The highest residual similarity has been detected between GalR1 and GalR2 (42%) as well as GalR3 (38%).

GalR1 mRNA distribution determination with RT-PCR showed its exclusive expression in the CNS and peripheral nervous system (PNS), where it was detected in hippocampus, hypothalamus, cortex, amygdala, spinal cord, and dorsal root ganglia (Waters & Krause 2000).

GalR1 is coupled to $G\alpha_{i/}o$. Its activation leads to inhibition of forskolin-stimulated cAMP production in a pertussistoxin (PTX)-sensitive manner (Wang *et al.* 1998, Habert-Ortoli et al. 1994, Waters & Krause 2000), which in turn leads to the opening of the G-protein-regulated inwardly rectifying K^+ (GIRK) channels (Lang *et al.* 2007). Furthermore, GalR1 stimulation increases PTX sensitive MAPK activity in a PKC independent manner (Wang *et al.* 1998).

1.3.2. Galanin receptor subtype 2

The second galanin receptor, GalR2, was identified from rat hypothalamus (Ahmad *et al.* 1998, Howard *et al.* 1997, Smith *et al.* 1997). The 387 amino acid protein is translated from the human GalR2 gene, which is comprised of 2 exons. As GalR1, the human GalR2 also has high sequence identity (87%) and similarity (92%) with rat GalR2 (Kolakowski *et al.* 1998). Studies show that the distribution of GalR2 is more wide spread in CNS and PNS than that of GalR1. High levels of GalR2 mRNA have been detected in hippocampus, hypothalamus, cortex, amygdala, spinal cord, DRG, anterior pituitary, lung, and kidney, while lower levels were seen in large intestine, spleen, heart, and liver (Waters & Krause 2000, Kolakowski *et al.* 1998).

In contrast to other galanin receptors, GalR2 signals through multiple classes of G proteins. Most commonly GalR2 acts through $G\alpha_{q/11}$. This pathway activates phosphilipase C (PLC) and leads to increased intracellular phosphoinositol release, mediating the release of Ca^{2+} into the cytoplasm from intracellular stores and opening Ca^{2+}-dependent channels (Kolakowski *et al.* 1998, Smith *et al.* 1997, Wang *et al.* 1998). GalR2 is also shown to couple to $G\alpha_o$-type G-protein and stimulate MAPK activity in protein kinase C (PKC) dependent, PTX-sensitive fashion (Wang et al. 1998). GalR2 is also proposed to activate RhoA in small lung cancer cells via coupling to $G_{12/13}$-type G-protein and enhance neuronal survival by suppressing caspase-3 and caspase-9 activity (Wittau *et al.* 2000, Ding *et al.* 2006). In several cell lines GalR2, similar to GalR1, has shown to inhibit adenylcyclase activity in a PTX-sensitive manner,

suggesting the activation of $G\alpha_i/\alpha_o$ types of G-proteins (Wang et al. 1998). Both GalR1 and GalR2 inhibit cyclic AMP-responsive element –binding (CREB) protein (Badie-Mahdavi *et al.* 2005).

1.3.3. Galanin receptor subtype 3

The third galanin receptor subtype, GalR3, was cloned from rat hypothalamic cDNA libraries (Wang *et al.* 1997a). The GalR3 gene is divided into two exons, which are translated into a 368 amino acid long protein (Kolakowski *et al.* 1998). The human GalR1 protein shares 36% and 58% homology with human GalR1 and GalR2, respectively, and 89% with rat GalR3 (Kolakowski *et al*. 1998).

GalR3 is suggested to have a more restricted expression pattern when compared to other two galanin receptors. Highest levels of GalR3 mRNA have been detected in the preoptic/hypothalamic area and in the subfornical organ (Mennicken *et al.* 2002). However, other studies report a wider distribution of GalR3 throughout the CNS and PNS (Wang et al. 1997a, Kolakowski *et al*. 1998).

Signaling properties of GalR3 are still vague. Similarly to GalR1, GalR3 appears to couple to $G_{i/o}$-type G-protein and inhibit AC, resulting in the opening of GIRK channels (Kolakowski et al. 1998, Smith *et al.* 1998).

1.4. Biological effects of galanin

Galanin is regulating numerous of pathological and physiological processes, including mood disorder, anxiety, alcohol intake in addiction, metabolic diseases, pain, and is expressed in some solid tumors through three different G-protein coupled receptors (Kyrkouli *et al.* 1986, Wiesenfeld-Hallin *et al.* 2005, Berger *et al.* 2004, Rada *et al.* 2004, Holmes *et al.* 2003b, Kuteeva *et al.* 2008a, Rodriguez-Puertas *et al.* 1997). The current study is focusing on the biological effects of galanin in two behavioral models, namely depression-like behavior and feeding.

1.4.1. Mood disorders and depression

Depression is estimated to be the fourth major cause of disability, affecting about 121 million people worldwide (Mathers *et al.* 2000, WFMH 2010). The lifetime prevalence of depressive episodes is estimated to be about 20% for men and 10% for women (DSM-IV 2000). Reduced social and work functions, indecisiveness, disturbance of sleep, reduced physical functions, low mood, feeling of hopelessness and worthlessness, sexual dysfunctions, and impairment of learning and memory, among many other symptoms have been related to mood disorders (Montgomery 2006, Clark *et al.* 2009, Eriksson *et al.* 2011). These abnormalities often result in reduced ability to work, pursuing personal interests and maintaining relationships. US workers suffering from major

depression were estimated to cause 36.6 billion dollars salary-equivalent productivity and 225.0 workdays lost per year (Kessler *et al.* 2006). Depression can cause serious consequences for the affected person and also to her/his family. It has been shown that around 60% of people who commit suicide have suffered from depression or from another mood disorder (Clark *et al.* 2009). However, despite all the serious consequences, less than 50% of all patients treated with currently available antidepressants show full remission (Crisafulli *et al.* 2011).

Understanding the aetiology of depression is still somewhat limited due its clinical heterogeneity. The most popular theory of depression, "the Monoamine Hypothesis" (Schildkraut 1965), has dominated this research field. It states that depression is the result of monoamines underactivity. NA and 5-HT are believed to be monoaminergic systems that are involved in the pathogenesis of mood disorders (Heninger *et al.* 1996, Weiss *et al.* 1981).

1.4.2. Galanin and depression-like behavior

Neuropeptides, including galanin, have been shown to play an important role in the regulation of mood disorders (Holmes *et al.* 2003a). Galanin is known to be co-localized with NA in LC and with 5-HT in dorsal raphe nucleus (DR). As mentioned above, both NA and 5-HT are implicated in the regulation of these disorders (Schildkraut 1965, Pieribone *et al.* 1995, Xu *et al.* 1998, Ma *et al.* 2001, Weiss et al. 1981, Heninger et al. 1996). Galanin has been shown to regulate serotonergic and noradrenergic system by having inhibitory actions on 5-HT/DR and NA/LC neurons (Pieribone et al. 1995, Xu *et al*. 1998, Ma *et al.* 2001). Inhibitory effects on noradrenergic system are supposedly mediated through GalR1/GalR3. The electrophysiological analysis using GalR1/2 agonist AR-M961 and GalR2 agonist AR-M1896 demonstrated that inhibition of firing and hyperpolarization of LC neurons is caused only by the GalR1/2 agonist, and not by the GalR2 agonist (Ma et al. 2001).

Modulation of serotonergic system is suggested to be similar to that of noradrenergic. Study using GalR2 agonist M1896 demonstrated that activation of GalR2 leads to increased 5-HT release, while GalR3 antagonist SNAP 37889 partially blocked the effect of i.c.v. injected galanin. Therefore, the inhibitory actions of galanin on serotonergic system are most likely mediated trough GalR1 and GalR3, whereas activation of GalR2 stimulates 5-HT release.

Accordingly, research using galanin receptor subtype specific ligands has demonstrated that GalR2 agonist AR-M1896 and systemically active GalR3 antagonist SNAP 37889 decreased immobility time in forced swim test (FST), while GalR2 antagonist M871, similarly with GalR1 agonist M617, had an opposite effect. Thus, suggesting that activation of GalR1 and GalR3 results in a depression like behavior, while stimulation of GalR2 leads to attenuation of the same disorder (Kuteeva *et al.* 2008b, Lu *et al.* 2005a, Swanson *et al.* 2005b, Barr *et al.* 2006).

1.4.3. Energy homeostasis

Energy homeostasis is a process in which the brain regulates the intake and the expenditure of the energy. This process is regulated by a complex endocrine system which includes both centrally and peripherally expressed peptides. Neuropeptides, that are involved in regulation of appetite, can be divided to orexigenic (appetite-stimulating) and anorectic peptides. NPY, melanin concentrating hormone (MCH), orexins A and B, agouti – related peptides, and galanin are known to stimulate food consumption, while alpha-melanocyte stimulating hormone (αMSH), corticotropin releasing hormone (CRH), cholecystokinin (CCK), cocaine and amphetamine regulated transcript (CART), neurotensin, glucagon-like peptide 1 (GLP 1), and bombesin are known to have anorectic actions (Tritos & Maratos-Flier 1999, Hillebrand *et al.* 2002). Therefore, understanding the central and peripheral factors of energy homeostasis might lead to effective treatments to control obesity, one of the leading causes of preventable death in western countries.

1.4.4. Galanin and feeding

Galanin plays a critical role in regulation of energy homeostasis. Direct administration of galanin into the lateral ventricle and the paraventricular nucleus (PVN) has been shown to robustly stimulate feeding in rodents (Crawley *et al.* 1990, Kyrkouli *et al.* 1986). The effect is higher in rodents fed with high fat diet than with regular diets (Tempel *et al.* 1988). In addition, a similar effect of galanin has also been detected in goldfish and chicks (Tachibana *et al.* 2008, de Pedro *et al.* 1995), suggesting that the orexigenic mechanism of galanin is conserved beyond animal species. Chronic administration of galanin to PVN resulted in the significant increase in daily calorie intake and body weight (Yun *et al.* 2005). Similar effect, increased fat-rich diet consumption (by 55%), has also been detected in galanin over expressing mice; while galanin knockout (KO) mice showed a decrease in fat rich diet ingestion (by 48%) (Karatayev *et al.* 2009, Karatayev *et al.* 2010). Furthermore, Adams and colleagues demonstrated that chronic administration of galanin by mini-osmotic pumps into lateral ventricle of galanin KO mice partially reversed the fat avoidance phenotype (Adams *et al.* 2008). Moreover, PVN injections of M40 (galanin receptor antagonist) decreased the intake of fat-rich diet (Leibowitz & Kim 1992), whereas co-administration of galanin antagonist with galanin blocked the galanin induced feeding effect (Corwin *et al.* 1993). Similarly, chronic PVN injections of antisense oligonucleotides to GAL mRNA in rats significantly reduced the daily intake of fat and weight gain (Akabayashi *et al.* 1994). However, previous research has shown that exogenous galanin only stimulates food consumption when food is readily available (Anderson *et al.* 2012). Taken together, these data indicate that galanin promotes overeating and weight gain by regulating fat intake when food is freely accessible.

Galanin is known to coexist with NA in brain of rats, including PVN, the region that is sensitive to galanin's stimulatory effect on feeding (Kyrkouli *et al.* 1990, Melander et al. 1986). Pharmacological studies using α_2-receptors blockers have shown to attenuate galanin induced feeding response in rodents (Kyrkouli *et al.* 1990) as well as in goldfish and chicks (de Pedro *et al.* 1995, Tachibana et al. 2008). Similar effect has been demonstrated using an opioid receptor antagonist naloxone (highest affinity towards μ-receptor), which reduced feeding evoked by i.c.v. administered galanin (Barton *et al.* 1995, Dube *et al.* 1994). Therefore, the orexigenic effect of galanin is suggested to be related to α_2-receptor and opioid μ-receptor.

Several studies indicate that leptin reduces galanin mRNA levels in PVN and thereby suppresses galanin induced food intake (Cheung *et al.* 2001, Sahu 1998), while the sensitivity of body weight and fat tissue to leptin is inhibited by galanin (Hohmann *et al.* 2003).

Evidence suggests that GALP, similarly to galanin, also stimulates feeding and gain in body weight. Although, the orexigenic action of i.c.v. injected GALP in rats is short, stimulating feeding only over the first hour, whereas in mice it had no effect (Seth *et al.* 2003). After 24h GALP reduced the food intake and body weight in both, mice and rats, while similar response was not seen when GalR2/3 agonist was administered. Thus, suggesting that GALP mechanism of action on feeding regulation might be partly independent of GalR1–3, and it may have its specific receptor that is not yet determined.

1.5. Galanin receptor ligands

All three galanin receptor subtypes, GalR1 – GalR3, are known to have somewhat different binding and signaling properties. Endogenous galanin is known to bind with a high affinity and act as an agonist on all three of them. Therefore, finding subtype specific ligands is of great interest in galanin field. However, despite the urgent need there are only few workable compounds available.

To date, the developed galanin ligands can be classified by their structure (peptide ligands and non-peptide ligands), by their ability to activate the receptor (agonist, antagonist or allosteric modulators), as well as by their ability to penetrate BBB.

1.5.1. Peptide ligands

In addition to endogenous galanin, several truncated galanin fragments are available (Table 5) (Wang *et al.* 1997b). Galanin fragment Gal(1–16) has shown to retain high affinity towards galanin receptors and to be highly efficient agonist both *in vitro* and *in vivo* (Wang et al. 1997b, Lu *et al.* 2005c).

A widely used peptide ligand ARM1896 (Gal (2–11)) was first published as GalR2 subtype specific agonist by Liu and colleagues in 2001 (Liu *et al.* 2001). However, they did not test it on GalR3 and following publications have shown that ARM1896 binds to GalR3 with a similar affinity as to GalR2 (Lu *et al.* 2005b).

The fact that GalR1 does not tolerate deletions on the N-terminal part of the galanin peptide has been used to design several other galanin fragments having preferential binding towards GalR2 when compared to GalR1 (Table 5) (Wang *et al.* 1997b).

Another endogenously occurring peptide, galanin-like peptide (GALP), was first demonstrated as a high affinity agonist for GalR1 and GalR2 with a 20 times better affinity towards GalR2 (Ohtaki *et al.* 1999). However, a recent study testing the binding affinity of GALP on all three galanin receptors showed that instead of GalR2 GALP has more than 10 times preferential binding towards GalR3 (Boughton *et al.* 2010).

Table 5. Affinities of galanin, GALP, and their fragments on three galanin receptor subtypes

Name	**GalR1**	**K_i (nM) GalR2**	**GalR3**	**Reference**
Rat galanin(1–29)	1.0	1.5	1.5	(Wang et al. 1997b)
	0.3 (h)	1.6 (h)	12 (h)	(Borowsky *et al.* 1998)
	0.9 (h)	1.2 (h)	7.4 (h)	(Lu et al. 2005c)
Human galanin(1–30)	0.4 (h)	2.3 (h)	69 (h)	(Borowsky et al. 1998)
Porcine galanin(1–29)	0.23 (h)	0.95 (h)	9.8 (h)	(Borowsky et al. 1998)
Galanin(1–16)	4.8	5.7	50	(Wang et al. 1997b)
Galanin(2–29)	85	1.9	12	(Wang et al. 1997b)
Galanin(3–29)	>1000	>1000	>1000	(Wang et al. 1997b)
Galanin(2–11)	>5000 (h)	88	271	(Lu et al. 2005b)
Porcine GALP	4.3	0.24	–	(Ohtaki et al. 1999)
Human GALP	77 [a] (h)	28 [a] (h)	10 [a] (h)	(Lang et al. 2005)
Human GALP(1–32)	129	69	–	(Lang et al. 2005)
Human GALP(3–32)	33	15	–	(Lang et al. 2005)
Rat GALP	45 [a]	18.7 [a]	1.53 (h) [a]	(Boughton et al. 2010)

Displacement is performed on the rat galanin receptor unless indicated otherwise.
(h) human; [a] presented as IC_{50} values; – not determined.

There are also several chimeric peptide ligands available. M15 (Bartfai et al. 1991), M35 (Ögren *et al.* 1992), C7, and M40 (Crawley *et al.* 1993) are all high-affinity galanin receptor antagonists. Although, when delivered i.c.v. or intrathecally (i.t.) at doses higher than 10 nmol all these ligands are known to have partial agonistic nature (Kask *et al.* 1995, Bartfai *et al.* 1993, Lu et al. 2005c). At lower doses (0.1 to 10 nmol), when delivered i.c.v. or i.t, they maintained their antagonistic properties (Lu et al. 2005c).

M15, often referred as galantide, was the first antagonist in the galanin field. This chimeric peptide ligand is composed of N-terminal galanin (1–13) fragment and of C-terminal substance P (5–11) fragment (Table 6–7). Displacement studies on the membranes from the rat tissues demonstrated around 10-fold higher affinity of M15 than endogenous galanin (Bartfai *et al.* 1991). However, M15 was chemically unstable and therefore a series of chemically more stable galanin receptor agonist were developed. Later binding studies have revealed that M15, M35, and M40 are non-specific towards different galanin receptor subtypes (Lu *et al.* 2005c) and this has limited their valuable contribution to delineate the galaninergic system. Still, these ligands have been very useful tools in the toolbox that has been used to elucidate the role of endogenous galanin in different systems, as well as in characterizing galanin receptor subtypes.

To address the ligand selectivity problem, two galanin receptor subtype selective ligands, M617 and M871, were developed. The design of M617 is based on the sequence of M35, where an additional amino acid residue Gln was added to position 14. This modification resulted in 25-fold selectivity towards GalR1 when compared to GalR2 and agonistic activity *in vitro* as well as *in vivo* (Lundström *et al.* 2005). The design of M871 in the other hand was based on M40, in which the N-terminal Gly was deleted and two extra amino acid residues were added to the sequence. These modifications resulted in a GalR2 antagonist (Sollenberg *et al.* 2006, Sollenberg *et al.* 2010).

Table 6. The sequences of previously published galanin receptor peptide based ligands

Name	Sequence
M15	GWTLNSAGYLLGPQQFFGLM-amide
M35	GWTLNSAGYLLGPPPGFSPFR-amide
M40	GWTLNSAGYLLGPPPALALA-amide
M617	GWTLNSAGYLLGPQPGFSPFR-amide
M871	WTLNSAGYLLGPEHPPPALALA-amide
Gal(2–11)	WTNLSAGYLL-amide
Gal-B2	(Sar)WTLNSAGYLLGPKKK(palmitoyl)K-amide
[N-Me,des-Sar]Gal-B2	(N-Me)WTLNSAGYLLGPKKK(palmitoyl)K-amide

While all these high affinity ligands are excellent tools to study the galaninergic system, they still exhibit several major drawbacks. First, they do not penetrate BBB, a major obstacle to deliver macromolecular entities, such as peptides, to CNS. Secondly, short half-lives and large volumes of distribution in the blood. In addition, they have weak selectivity towards different galanin receptor subtypes.

In year 2008, a series of systemically active galanin ligands was introduced. In this study cationic amino acid residues and a palmitoyl moiety were used to increase the bioavailability and the ability to penetrate BBB of the ligands. The most effective analogue Gal-B2 (NAX 5055) exhibited slight selectivity towards GalR1 when compared to GalR2 and displayed anticonvulsant activity in several different animal models (Bulaj *et al.* 2008, White *et al.* 2009). Later, Gal-B2 was redesigned yielding a GalR2 preferring analogue with an 18-fold better affinity over GalR1. Similarly to Gal-B2, the novel analogue also exhibited anticonvulsant activity following systemic delivery (Robertson *et al.* 2010).

Table 7. The affinities of previously published peptide based ligands for galanin receptors

Name	**GalR1**	**K_i (nM) GalR2**	**GalR3**	**Reference**
M15	0.65	1.0	1.0	(Smith et al. 1998)
M35	0.11 (h)	2.0 (h)	–	(Borowsky et al. 1998)
	0.325	3.24	2.09	(Smith et al. 1998)
	4.8	8.2	4.7	(Lu et al. 2005c)
M40	2.4 (h)	4.1 (h)	–	(Borowsky et al. 1998)
	6.76	3.55	79.4	(Smith et al. 1998)
	1.8	5.1	63	(Lu et al. 2005c)
M617	0.23 (h)	5.7 (h)	–	(Lundström et al. 2005)
	–	–	49 (h)	(Sollenberg et al. 2010)
M871	420 (h)	13 (h)	–	(Sollenberg et al. 2006)
	–	–	> 10000 (h)	(Sollenberg et al. 2010)
Gal(2–11)	>5000(h)	88	271	(Lu et al. 2005b)
Gal-B2	3.5 (h)	51.5 (h)	–	(Bulaj et al. 2008)
[N-Me,des-Sar]Gal-B2	364 (h)	20 (h)	–	(Robertson et al. 2010)

Displacement was performed on the rat galanin receptor unless indicated otherwise. (h) human; – not determined.

1.5.2. Non-peptide ligands

Structure–activity relationships (SAR) studies have demonstrated that Trp-Asn-Tyr in positions 2, 5, and 9 are important pharmacophores of galanin. Based on this information small chemical libraries were designed. Screening of the libraries revealed two systemically active compounds galnon (Saar *et al.* 2002) and galmic (Ceide *et al.* 2004). Galnon was introduced in year 2002, and is shown to act as a high affinity agonist for all galanin three receptor subtypes (Saar et al. 2002). It's *in vivo* activity has been demonstrated in multiple animal models, including feeding (Abramov *et al.* 2004), anxiety and depression (Rajarao *et al.* 2007), epileptic seizure (Saar *et al.* 2002), and pain (Wu *et al.* 2003). Galmic is shown to be GalR1 subtype specific ligand, although it has yet not been tested on GalR3 (Ceide *et al.* 2004). Despite its somewhat high molecular weight it penetrates BBB relatively quickly and is 6–7 times more potent in inhibiting self-sustaining status epilepticus (SSSE) than galnon (Bartfai *et al.* 2004). In addition, it has been demonstrated that galmic attenuates the immobility time of rats in FST (Bartfai *et al.* 2004). However, galmic and galnon have several major drawbacks. First, they have low affinity towards galanin receptors. Furthermore, it has been observed that at concentration of 10 μM they interact with multiple important targets in CNS, i.e. 5-HT1A, D2 dopamine, Ghreline receptors (Lu et al. 2005c).

Table 8. The affinities of previously published non-peptide based ligands for galanin receptors

Name	GalR1	K_i (nM) GalR2	GalR3	Reference
Galnon	11700	34100	–	(Saar et al. 2002)
Galmic	34200	>100000	–	(Bartfai et al. 2004)
Sch 202596	1700 [a]	–	–	(Chu *et al.* 1997)
Dithiepine-1,1,4,4-tetroxide	190 [a]	>30000 [a]	–	(Scott *et al.* 2000)
SNAP 37889	>10000	>10000	17.4	(Swanson *et al.* 2005a)
SNAP 398299	>1000	>1000	5.3	(Swanson et al. 2005a)
GalRant	>10000	>10000	15	(Barr et al. 2006)

Displacement was performed on the rat galanin receptor unless indicated otherwise.
[a] presented as IC_{50} values;–not determined.

Random screening of large chemical libraries revealed couple of novel non-peptide antagonist for galanin. Sch 202596, a GalR1 antagonist, was discovered from the fermentation of a fungal culture *Aspergillus* sp. isolated from the

tailing piles of an abandoned uranium mine in Tuolemene County, California. Sch 202596 exhibited inhibitory effect on GalR1 with an IC_{50} value of 1.7 μM (Table 8) (Chu *et al.* 1997). Another galanin receptor antagonist, 2,3-dihydro-2-(4-methylphenyl)-1,4-dithiepin-1,1,4,4-tetroxide, detected from corporate compound collection demonstrated an IC_{50} value of 190 nM for GalR1 (Scott et al. 2000). However, its chemical reactive nature and solubility made this compound unattractive from a drug discovery point of view. In addition, both mentioned antagonist are low affinity compounds and therefore have not been tested in multiple animal models.

In 2006, Konkel and colleagues published a series of 3-imino-2-indolones acting as high affinity antagonist for GalR3. One of them, 1,3-dihydro-1-phenyl-3-[[3-(trifluoromethyl)phenyl]imino]-2*H*-indol-2-one (SNAP37889) was shown to have a K_i value of 17 nM (Table 8) (Konkel *et al.* 2006a). However, the published indolones demonstrated very poor aqueous solubility. Therefore, a series of amino analogues of 1,3-dihydro-1-phenyl-3-[[3-(trifluoromethyl)phenyl]imino]-2*H*-indol-2-one were synthesized in order to increase the solubility and still retain the high binding affinity. This work resulted in a compound with an increased aqueous solubility and selectivity, 1,3-dihydro-1-[3-(2-pyrrolidinylethoxy)phenyl]-3-[[3-(trifluoromethyl) phenyl] imino]-2H-indol-2-one, often referred as SNAP398299 (Konkel *et al.* 2006b, Swanson et al. 2005a). Both, SNAP398299 and SNAP37889, have been effectively tested in various *in vivo* models. Later, Barr and colleagues introduced another indolone based GalR3 antagonist, 3-(3,4-dichloro-phenylimino)-1-(6-methoxypyridin-3-yl)indolin-2-one, and also demonstrated its activity in preclinical tests (Barr *et al*. 2006). Taken together, these studies suggest that GalR3 antagonist have anxiolytic- and antidepressant-like effects (Swanson *et al.* 2005a, Barr *et al.* 2006).

In 2010, the first GalR2-positive allosteric modulator CYM2503 was characterized in The Scripps Research Institute (Lu *et al.* 2010). CYM2503 was demonstrated to stimulate IP production in HEK293 cells stably expressing GalR2 receptors, while showing no detectable affinity for GalR2. CYM2503 exhibited anticonvulsant activity upon i.p. administration in acute seizure models in rats and mice. One year later, in the same institute small molecular 2,4,6-triaminopyrimidine derivatives were designed and tested on GalR1 and GalR2. A series of them were shown to bind GalR2 with an IC_{50} value ranging from 0.33 to 1.0 μM (Sagi *et al.* 2011).

2. AIMS OF THE STUDY

Neuropeptide receptors are currently of high interest as a class of drug targets for the pharmaceutical industry. However, the precise role of different galanin receptor subtypes in various human diseases and pathological conditions is still not fully understood due to the lack of galanin receptor subtype specific ligands. Therefore, the research presented in this thesis is mainly focusing on the development of novel subtype specific ligands, and evaluating their biological effect in behavioral models. The main aims of the respective papers are presented below:

Paper I. The main objective in this paper is to develop a novel GalR2 subtype specific ligand, and to introduce a stable hGalR3 cell line that is useful for the characterization of future galanin ligands.

Paper II. The aim of this paper is to introduce several novel GalR2 selective agonists, and to compare the most selective one with a known GalR1 selective agonist M617 for their ability to stimulate acute consumption of several foods.

Paper III. The objective within this publication is to develop a novel highly subtype specific GalR2 agonist, and to compare its ability with an antidepressant medication imipramine to attenuate depression-like behavior in tail suspension test.

Paper IV. The aim of this study is to design an approach to increase *in vivo* usability of peptide ligands, and to develop a series of systemically active novel GalR ligands with preferential binding towards GalR2. Furthermore, the ability of the novel ligands to attenuate depression-like behavior in several different animal models is compared with a common clinically used antidepressant medication imipramine. Moreover, Paper IV includes an in-depth characterization of one of these peptide ligands.

3. METHODOLOGICAL CONSIDERATIONS

3.1. Solid phase peptide synthesis

All peptides used in this thesis were synthesized using solid phase peptide synthesis (SPPS) . The introduction of the SPPS technique by Bruce Merrifield in 1963 was a big breakthrough in peptide synthesis methodology (Merrifield 1963). It dramatically changed the strategy of peptide synthesis and simplified the steps of purification. The principle of SPPS is based on repeated cycles of coupling and deprotection of protected amino acids, carried out on an insoluble support, resin.

The peptides that have been used within this thesis were synthesized using small scale (0.1 mmol) 9-fluorenylmethyloxycarbonyl (Fmoc) solid-phase peptide synthesis strategy on an automated peptide synthesizer (Applied Biosystems™ model 433A). Fmoc amino acids were coupled as hydroxybenzotriazole (HOBt) esters to a *p*-methylbenzylhydrylamine (MBHA) resin (Fluka), which will generate a C-terminally amidated peptide after the final cleavage. The peptides were finally cleaved from the resin using 95% TFA (trifluoroacetic acid), 2.5% TIS (*triisopropylsilane),* and 2.5% H_2O solution for 3 h.

To obtain branched peptides (Paper II and IV) with Glu or stearic acid substitution amino acid residue Fmoc-Lys(Mtt)-OH (Iris Biotech GMBH, Germany) was used. 4-methyltrityl(Mtt) – protecting group was removed manually by using 1% TFA, 2% TIS solution in DCM (dichloromethane). Stearic acid was coupled as a hydroxybenzotriazole (HOBt) ester overnight.

All the peptides were purified by reverse-phase HPLC using Nucleosil 120–3 C-18 column (SP 250/10 Nucleosil Macherey-Nagel, Germany), and the correct molecular weight was determined by Perkin Elmer prOTOF™ 2000 matrix assisted laser desorption ionization time-of-flight mass spectrometry (Perkin Elmer, Sweden).

3.2. Galanin receptor ligands (Papers I–IV)

In paper I, the design of M1145 is based on a previously published chimeric ligand M871, the GALP peptide and its preferences towards GalR2, and the fact that the deletion of the Gly residue on the N-terminus of galanin yields in selectivity towards GalR2. For M1145, the galanin sequence 1–13 was elongated with six amino acid residues from the GALP sequence, four amino acid residues N-terminally (RGRG) and two amino acid residues C-terminally (VL). The Gly residue in the N-terminus of galanin (deleted in M871) was replaced with an Asn to disturb binding towards GalR1 and potentially also to GalR3. M1145 also contains the C-terminal portion of the GalR2 selective ligand M871; Pro-Pro-Pro-(Ala-Leu)$_2$-Ala and the C-terminal amide (Sollenberg *et al.* 2006, Ohtaki *et al.* 1999).

In paper II, we evaluate M1151, a previously published peptide by Pooga and colleagues in a study where they introduced a series of peptide ligands with different compounds that have been added orthogonally to the side chain of Lys residue in position 14 (Pooga *et al.* 1998). In addition, we also introduce two novel peptide ligands, M1152 and M1153. Both ligands are identical with M1151 in the C-terminal part of the sequence, but have different modifications in the N-terminus. In M1152, similarly with M871, the Gly residue in position 1 is deleted, whereas in M1153, in analogy with M1145, the N-terminal part is elongated with four amino acids (RGRG) and the Gly in the N-terminus is substituted with Asn (Sollenberg *et al.* 2006, Runesson *et al.* 2009). All these modification were introduced in order to improve the selectivity of the ligands towards GalR2.

Paper III demonstrates a highly subtype specific GalR2 ligand M1160. The design of M1160 is based on the sequence of M1145 (Runesson *et al.* 2009), in which the amino acid residue Thr was deleted from the position 6 in order to decrease binding towards GalR3. The relevance behind this strategy is based on previous research indicating that Thr plays an important role when binding to GalR3, but is not crucial when binding to other GalR subtypes (Runesson *et al.* 2010b, Church *et al.* 2002, Kask *et al.* 1998, Lundström *et al.* 2007).

In paper IV, we introduce a series of systemically active novel GalR ligands (J17-J21) with preferential binding towards GalR2. The design of these ligands was based on M1145 and M1153 (Runesson *et al.* 2009, Saar *et al.* 2011). Similarly to M1153 and M1145, the N-terminus of J17-J21 was elongated with four amino acids residues (RGRG) from GALP sequence and the Gly in position 5 was substituted with Asn (Lundström *et al.* 2007, Runesson *et al.* 2009, Saar *et al.* 2011). In addition, to increase the pharmacokinetic properties and the CNS activity of the ligands a stearoyl acid was coupled to the side chain of the Lys in the C-terminal part of the peptides. Furthermore, the C-terminal part was elongated with additional positively charged amino acids. In the peptide J18, the three C-terminally positioned L-Lysins were substituted to D-Lysins in order to reduce enzymatic degradation and further increase the bioavailability.

3.3. Radioligand binding assay (Paper I–IV)

Radioligand binding assays are often used in pharmacological research in order to study receptor systems. In this thesis, competitive binding assay was performed to screen the binding properties of novel galanin receptor ligands to their target receptors, GalR1-GalR3. In this assay, the concentration of the radioligand is held constant throughout the experiment, while various concentrations of the ligand are added. Thereafter, the ability of the radioligand to bind the receptor in the presence of the unlabeled ligand is measured and the IC_{50} is calculated. IC_{50} presents the concentration of the unlabeled compound

where it produces 50% of the specific binding of the radioligand. However, the IC_{50} value depends on the radioligand concentrations that might vary between experiments. Therefore, this value is converted using Cheng-Prusoff equation into K_i value.

Porcine ^{125}I-galanin was used as the radioligand. It is the most commonly used radioligand in field, since radiolabeling of galanin is performed through the iodiation of Tyr, and porcine galanin is the only known endogenous galanin sequence that possesses an extra Tyr. Although human ^{125}I-galanin is available, it is rarely used due to its labeling of Tyr in position 9, the residue that is known to be important for ligand-receptor interactions (Land *et al.* 1991a).

Throughout this thesis silanized plastic was used to minimize the binding of the peptides to the plastic. Additionally, polyethyleneimine (PEI) was used to minimize adsorption to the glass fiber in order block unspecific binding.

3.4. Inositol phosphate accumulation (Paper I–III)

Previous research has shown that GPCR G_q stimulation leads to activation of phospholipase c (PLC) and triggers inositol phosphate cascade. Therefore, inositol phosphate (IP) accumulation assay can be used to measure the activation of G_q-proteins. The assay is based on the detection of tritium labeled IP that is accumulated in the cells following the stimulation of the G_q receptor. Despite the fact that this protocol is relatively insensitive and time consuming the method has been widely used in galanin research.

In this assay, the cells were labeled with ^{3}H-myoinositol, a radiolabel which incorporates into phosphatidylinositol 4,5-bisphosphate (PIP_2) and thereafter into its hydrolyzed product IP_3 and its downstream metabolics. IP recycling into myo-inositol was blocked by adding Lithiumchloride (LiCl). The inositol phosphates, including IP_3 and its downstream metabolics inositol 4,5-bisphosphate (IP_2) and IP, were extracted in the presence of perchloric acid and separated by an anion exchange column. The total radioactivity was measured by a β-counter and normalized to the total count in each sample.

3.5. Depression-like behavior (Paper III–IV)

Since depression is a complex disorder, it is very difficult to develop an animal model that would be able to mimic all the symptoms of depressed patients. Moreover, some of the symptoms are impossible to model in rodents. However, before starting complex and expensive preclinical trials the initial screening of novel compounds with antidepressant properties requires a simple and rather robust model. Therefore, several test procedures have been developed, including the tail suspension test (Steru *et al.* 1985), the forced swim test (Porsolt *et al.* 1977), the learned helplessness model (Seligman & Maier 1967), and the chronic mild stress (Willner 1997). Within this thesis, two of the mentioned

models, the forced swim test (FST) and the tail suspension test (TST), were used to test novel galanin receptor subtype specific ligands for their ability to attenuate depression-like behavior, and to show their agonistic properties *in vivo*. Both, FST and TST, are based on the same principle: animals are subjected to a short-term inescapable period of stress and the time of immobility is measured.

3.5.1. Tail suspension test

For this test, mice were individually suspended to the wooden beam by the tail, using adhesive tape that has been applied 1 cm from the end of the tail, for a 6-minute period. The duration of immobility during the last 4 minutes was measured. Immobility was defined as complete lack of movements beside respiration.

3.5.2. Forced swim test

The FST consisted of one episode of 6 minutes swimming (glass container, diameter 15 cm, height 30 cm), of which the last 4 minutes were scored for total immobility time. Similarly to TST, the duration of immobility during the last 4 minutes was measured. Immobility was defined as complete lack of movements beside respiration.

4. RESULTS AND DISCUSSION

All four studies presented within this thesis are focusing on the development of novel galanin receptor subtype specific ligands. Besides, the first study also presents the development and characterization of an inducible GalR3 cell line. In the second study the ability of GalR1 and GalR2 agonists to stimulate acute food consumption in mice has been compared, whereas the focus in the third paper consisted in demonstrating the ability of a novel GalR2 agonist (M1160) to attenuate depression-like behavior in TST. Finally, the fourth study presents an approach on how to increase the *in vivo* usability of peptide ligands. It also demonstrates the ability of several novel systemically active GalR2 preferring ligands to attenuate depression-like behavior in a side by side comparison with the clinically used antidepressant imipramine in different animal models.

4.1. Novel galanin subtype selective ligands (Paper I–IV)

The importance of galanin receptor subtype specific ligands in delineating the galanin system cannot be overestimated. As the galaninergic system remains a promising drug target, the information about galanin receptor subtype mediated effects is of high importance. The main objective of this thesis is to develop tools that contribute to the characterization of the galaninergic system, thus designing and characterizing novel GalR2 subtype specific ligands using various approaches.

Paper I demonstrates the design and characterization of a novel galanin analogue M1145 with selectivity towards GalR2, compared to GalR1 and GalR3 (Table 10). The design of M1145 was based on extending the N-terminal part of the galanin analogue. In addition, this study also examines the signaling of M1145 through G_q, the preferential G-protein for the GalR2. M1145 showed increased IP production in a similar manner with galanin (Table 9). Furthermore, M1145 also demonstrated an additive effect on signal transduction in the presence of galanin. Taken together, Paper I presents a novel GalR2 subtype specific ligand with agonistic properties.

Paper II presents one previously published and two novel (M1152 and M1153) galanin receptor subtype specific ligands. This design of the C-terminal part of the peptides was based on an article by Pooga and colleagues, where several different compounds were orthogonally added to the side chain of the Lys residue situated at position 14 and tested for binding affinity on receptors isolated from rat hypothalamic tissues (Pooga *et al.* 1998). In contrast to Pooga`s paper, the binding assays in this current study were performed on membranes from three different cell lines, each expressing a single galanin receptor subtype. This was done in order to examine the subtype specificity of the ligands.

Table 9. Galanin receptor ligand studied in this thesis

Name	Sequence
Rat galanin	GWTLNSAGYLLGPHAIDNHRSFSDKHGLT-amide
M1145	RGRGNWTLNSAGYLLGPVLPPPALALA-amide
M1151	GWTLNSAGYLLGPK(ε-NH-C(O)(CH2)$_2$C(NH$_2$)COOH)-amide
M1152	WTLNSAGYLLGPK(ε-NH-C(O)(CH$_2$)$_2$C(NH$_2$)COOH)-amide
M1153	RGRGNWTLNSAGYLLGPK(ε-NH-C(O)(CH$_2$)$_2$C(NH$_2$)COOH)-amide
M1160	RGRGNWLNSAGYLLGPVLPPPALALA-amide
J17	RGRGNWTLNSAGYLLGPKKK(εNH·C(O)stearic acid)-amide
J18	RGRGNWTLNSAGYLLGPkkK(εNH·C(O)stearic acid)k-amide
J19	RGRGNWTLNSAGYLLGPOOK(εNH·C(O)stearic acid)O-amide
J20	RGRGNWTLNSAGYLLGPXXK(εNH·C(O)stearic acid)K-amide
J21	O^{1}GO^{1}GNWTLNSAGYLLGPO^{1}O^{1}K(εNH·C(O)stearic acid)O^1-amide

X – 6-aminohexanoic acid. O^1 – Ornithine

M1151(Pooga *et al.* 1998) demonstrated rather unselective binding properties with somewhat preferential binding towards GalR2 when compared to GalR1 and GalR3 (Table 19). Therefore, to improve the preferential GalR2 binding M1151 was modified either by extension of the N-terminus (M1153) or by deletions in the N-terminus (M1152). The deletion of the N-terminal Gly residue in M1152 in analogy with previously designed M871 (Sollenberg *et al.* 2006) resulted in increased subtype selectivity, but also in a two-fold lower affinity towards GalR2. The design of M1153 was based on the previously published M1145 (Runesson *et al.* 2009). Similarly to M1145, the N-terminal part of M1153 was elongated with four amino acid residues (RGRG) from the galanin-like peptide (GALP) sequence (Ohtaki *et al.* 1999, Runesson *et al.* 2009), and the N-terminal Gly was substituted with an Asn in order to disturb binding to GalR1 and GalR3 (Runesson *et al.* 2009, Sollenberg *et al.* 2006). M1153 exhibited a noticeably higher selectivity than previously published ligands for GalR2, and still retained high binding affinity, comparable with the affinity of galanin, for GalR2. One could speculate that the improved binding affinity of M1153 could be explained by the possibility that Glu in the side chain of Lys can form additional hydrogen bonds, which may contribute to the interactions with GalR2. The observed lower binding affinity of M1151, M1152, and M1153 towards GalR3 could be due to the orthogonally coupled Glu, since GalR3 has been proposed to have a relatively narrow binding pocket compared to GalR2 (Runesson *et al.* 2010a, Jurkowski *et al.* 2013).

Table 10. The affinities of novel ligands presented in this thesis

Name	GalR1	K_i (nM) GalR2	GalR3	K_i (GalR1) / K_i (GalR2)	K_i (GalR3) / K_i (GalR2)
Rat galanin	1.75 [a]	2.98 [a]	4.49 [a]	0.6	1.5
M1145	587 [a]	6.55 [a]	497 [a]	90	76
M1151	98.6 [b]	28.9 [b]	874 [b]	3.4	30
M1152	2370 [b]	36.4 [b]	656 [b]	65	18
M1153	1890 [b]	4.98 [b]	230 [b]	380	46
M1160	15500 [c]	33.3 [c]	> 10000 [c]	465 [c]	>1000 [c]
J17	156 [d]	29 [d]	125 [d]	5.4 [d]	4.3 [d]
J18	138 [d]	20 [d]	112 [d]	6.9 [d]	5.6 [d]
J19	231 [d]	83 [d]	114 [d]	2.8 [d]	1.4 [d]
J20	25 [d]	4.9 [d]	13 [d]	5.1 [d]	2.7 [d]
J21	138 [d]	41 [d]	65 [d]	3.4 [d]	1.6 [d]

[a] Paper I, [b] Paper II, [c] Paper III, [d] Paper IV

As in paper I, the agonistic effect of M1153 was assessed by measuring IP accumulation. M1153 exhibited a clear dose dependent increase in IP production, whether administered alone or together with galanin.

Paper III demonstrates a highly subtype specific GalR2 ligand M1160 with ~500-fold selectivity (Table 10). Design of M1160 was based on M1145 (Paper I) and previously published mutagenesis studies indicating that Thr has an important role to play in binding to GalR3, but not to such a degree when binding to other two galanin receptor subtypes. The proposed theory states that Thr forms hydrogen bonds and stabilizes the binding conformation through vdW interactions when binding to GalR3 (Kask *et al.* 1998, Runesson *et al.* 2010a, Church *et al.* 2002, Lundström *et al.* 2007). The deletion of Thr from the 6th position of M1145 sequence abrogated the ability of M1160 to bind GalR3 at all. In addition, it also resulted in a tremendous loss of binding affinity towards GalR1 (Table 10). The truncation of Thr also led to a small loss of affinity towards GalR2, although still showing a relatively high K_i value towards GalR2. Similarly to M1145 (Paper I) and M1153 (Paper II), M1160 also expressed agonistic properties *in vitro*, which were assessed by its ability to stimulate IP production. A dose-dependent additive effect of signal transduction in the presence of 10 nM galanin was also seen. Taken together, this study presents a GalR2 subtype specific agonistic ligand that compared to previously published ligands such as M1153, galanin(2–11), and M1145 exhibited noticeably higher selectivity.

As suggested earlier, peptide mimics have a number of disadvantages when considering their drugability. First, their short half-lives due to enzymatic degradation and large volumes of distribution in the blood are a concern. Secondly, blood brain barrier (BBB) is a major obstacle when delivering macromolecular entities, such as peptides, to CNS. Paper IV addresses these issues by presenting an approach to increase *in vivo* usability of peptide ligands and demonstrates a series of systemically active novel GalR ligands, J17-J21 with preferential binding towards GalR2. The design of these ligands was based on two previously published GalR2 agonists M1145 and M1153. The N-terminal part of the novel ligands were elongated with the same four amino acid residues (Arg-Gly-Arg-Gly) that are present in the galanin-like peptide (GALP), as was done in case of M1145 and M1153. In order to disturb the ligand binding to GalR1 and GalR3 the N-terminal Gly in position 5 was replaced with Asn. To improve the pharmacokinetic properties (i.e. stability to degradation) and the CNS activity of the ligands, two commonly known strategies were used. First, the ligands were elongated with additional positively charged amino acids (cationization) (Witt & Davis 2006). Secondly, a stearic acid was coupled to the side chain of the C-terminal Lys of the ligands (lipidization) (Dasgupta *et al.* 2002). Both cationization and lipidization have shown to enhance the bioavailability of the peptides in the brain (Bulaj *et al.* 2008). Additionally, several L-amino acids in one of the ligands (J18) were replaced with D-amino acids to reduce enzymatic degradation and further increase the bioavailability. These modifications resulted in a slight loss of subtype specificity for GalR2 and in a decrease of binding affinity when compared to previously published GalR2 agonists M1145 and M1153 (Table 10). Nevertheless, all the tested peptides still exhibited a noticeable preferential binding towards GalR2. Furthermore, the biodistribution comparison of J18 with a previously published peptide M1145 clearly showed a much higher half-life of J18, which reflects in its higher stability to degradation. The levels of J18 were higher in all organs 1h and 3h post-injection in mice. At 24h post-injection, J18 had been cleared out from the organism, while M1145 had already been largely eliminated after 3h. In addition, the levels of J18 in the brain were 2.5 fold higher than that of M1145. These results suggest that the combination of increased stability and lipofilicity improved the ability of J18 to penetrate the BBB.

As mentioned before, ligands presented in Paper IV have somewhat decreased binding affinity and lower receptor subtype selectivity when compared to their forerunners. However, this may not always indicate anything about the usefulness of the peptides *in vivo*, because increased stability might compensate for lower affinity. Although, establishing high subtype selectivity ligands in conjunction with high binding affinity is of great interest.

4.2. Galanin in feeding regulation

As described previously, galanin plays a critical role in regulation of energy homeostasis. Previous research has shown that galanin has an immediate and robust effect on stimulation of food intake following the administration into the ventricle or PVN (Crawley *et al.* 1990, Kyrkouli *et al.* 1986, Kyrkouli *et al*. 1990, Leibowitz 2005). All three galanin receptor subtypes can be found in the hypothalamic region (Waters & Krause 2000), and therefore can mediate this effect. Paper II provides the first side-by-side comparison of highly galanin receptor-selective agonists on feeding behavior. Two of the evaluated subtype selective agonists M1153 and M1145 were shown to have no effect on the acute consumption of two highly palatable foods. In contrast, the GalR1 selective agonist M617 notably stimulated acute consumption of high-fat milk. These results are in concordance with previous research suggesting that galanin increases food intake through GalR1 (Lundström *et al.* 2005). Another study reported that galanin fragment Gal(2–29) has no effect on feeding, which is consistent with the results in the present study. Moreover, GalR1 knockout mice showed abnormal adaptation responses to dietary challenges of high fat and high glucose conditions, while normal regulation was seen on low fat and chow diets (Holmes *et al.* 2003b, Zorrilla *et al.* 2007, Wrenn *et al.* 2004). GalR2 knockout mice on the other hand demonstrated no differences in feeding behaviors or body weights (Gottsch *et al.* 2005). In summary, Paper II, in concordance with previous research, indicates that the GalR1 is most involved in the regulation of feeding.

4.3. Galanin in depression-like behavior

Galanin is known to play an important role in the regulation of mood disorders. Previous research has shown that different galanin receptor subtypes have distinct functions in regulating depression. Activation of GalR1 and GalR3 is suggested to result in a depression-like behavior, while stimulation of GalR2 leads to attenuation of the same behavior (Kuteeva et al. 2008a). Based on this information Paper III and IV test the potential agonistic properties of novel peptide ligands *in vivo* by using different animal models in order to evaluate their effects on antidepressant behavior.

Although previous research has shown evidence that stimulation of GalR2 leads to attenuation of depression-like behavior, Paper III is the first study demonstrating the same effect using a highly GalR2 subtype specific ligand. In this study TST, a common rodent model for depression-like behavior, was used to evaluate the effects of the tested compounds on antidepressant behavior. Both, M1160 and Imipramine, presented significant decrease in the immobility time when compared to the saline group. In summary, Paper III demonstrates agonistic and antidepressant properties of M1160.

Within Paper IV the involvement of systemically active GalR2 subtype preferring ligands in depression like-behavior were assessed in two different animal models (TST and FST) using different administration techniques. I.v. administration of several peptide ligands reduced the immobility time in FST. In the current study the highest effect was obtained by the peptide J18. It was comparable to the effect attained by the clinically relevant antidepressant imipramine. Remarkably, the effective doses of J18, 0.5 mg/kg and 0.25 mg/kg are equal to 192 nmol/kg and 96 nmol/kg, which reflect the strikingly high potency of the peptides.

As mentioned above, another model (TST) was used to further confirm the systemic activity of the novel peptides. As expected, similarly to the effect in FST, J18 exhibited potent systemic activity in TST. In addition, Paper IV shows that the antidepressive effect of J18 is also retained via the i.p. administration route, which is of high importance for animal studies, as it permits easy, stress-free handling and enables an experimental setup with chronic administration. Antidepressive drug screening animal models utilize acute administration of a drug (2 injections for rats and 1 injection for mice in TST or FST). However, it is generally known that chronic administration for 2–4 weeks is required for SSRIs to see antidepressive effect in clinics. Therefore, this current study investigated the possibility to use J18 in other depression models, which utilize repeated administration of a drug, because different feedback mechanisms or receptor desensitization may decrease the pharmacological potency of the compound. Chronic administration of J18 still retained its antidepressant-like effect in TST, suggesting that further characterization of J18 and other currently presented peptides in different models is possible.

Furthermore, to verify that galanin receptors mediate all the effects seen with J18, a non-selective galanin receptor antagonist M35 (i.c.v) was used concomitantly with J18 (i.p.). In agreement with our hypothesis, M35 blocked the effect induced by i.p. injected J18.

Taken together, Paper III and IV demonstrate *in vivo* agonistic properties of a series of GalR2 subtype preferring ligands. In addition, in accordance with previous research these current studies confirm that stimulation of GalR2 leads to attenuation of depression-like behavior. Previously, Kuteeva and colleagues tested GalR2(R3) agonist AR-M1896 and GalR2 antagonist M871 to demonstrate GalR2 involvement in depression-like behavior in FST. AR-M1896, similarly with fluoxetine, showed decreased values in immobility time, and M871 increased the immobility time in the FST (Kuteeva *et al.* 2008b). Similar results have been obtained using galanin antagonist to block the effect of fluexetine in FST, suggesting that the effect of fluoxetine treatment at least partially involves galanin related transmission (Lu *et al.* 2005a).

However, as depression is a complex and multifactorial disorder, more studies have to be carried out in order to confirm the antidepressant like actions of these ligands. Several different and more complex behavioral models of

depression should be tested in parallel with measurements of other functions that are confounds in the models of depression.

4.4. Development of GalR3 inducible cell line

Paper I introduces the development of a novel GalR3 inducible cell line, a well appreciated tool expanding the possibilities of characterizing galanin receptor ligands.

More specifically, a stable Flp-In T-Rex cell line with an inducible expression of hGalR3 was developed and characterized. Western Blot analysis confirmed a clear induction of the GalR3 expression after 24 and 48 h treatment with tetracycline, while showing no expression of GalR3 protein in non-induced cells. Displacement binding studies on GalR3 expressing membranes using galanin and a GalR3 selective antagonist SNAP37889 demonstrated their ability to inhibit ^{125}I-galanin binding, thus confirming the presence of GalR3 receptor. Furthermore, in the functional GTPγ assay on GalR3 expressing membranes rat galanin stimulated GTPγ activity over 75% of the basal activity. In the same setting SNAP37889 exhibited no effect, but inhibited dose-dependently the GTPγ activity induced by 1 μM galanin. Additionally, homologues competitive binding calculations showed that the protein expression level in Flp-In T-Rex GalR3 membranes corresponds to two other cell systems used in this thesis, indicating that the cell systems used in the current study are highly compatible.

Taken together, Paper I presents a novel GalR3 inducible cell line, which functional properties are in line with previous studies performed on human GalR3 (Branchek *et al.* 2000).

5. CONCLUSIONS AND FUTURE PROSPECTS

The main conclusions of the four papers comprising this thesis are presented below.

Paper I. A novel GalR2 subtype specific peptide M1145 was designed and characterized. It exerted agonistic properties *in vitro* by assessing its ability to stimulate IP production. In addition, a stable GalR3 cell line was developed, providing a new tool for characterization of future galanin ligands.

Paper II. Extension of the N-terminal part and replacement of a N-terminal Gly with Asn leads to higher receptor subtype specificity (M1153). Side by side comparison of GalR1 and GalR2 agonists suggests that galanin induced increased food intake is mediated through GalR1.

Paper III. Deletion of amino acid residue Thr from position 6 of M1145 (Paper I) led to a discovery of a highly subtype specific GalR2 ligand with agonistic properties. Additionally to *in vitro* studies, i.c.v. administered M1160 also demonstrated agonistic properties *in vivo* by attenuating depression-like behavior in a similar manner with Imipramine.

Paper IV. Introduction of a stearoyl moiety and additional positively charged amino acids to the C-terminal part of the M1153 (Paper II) sequence resulted in a series of GalR2 subtype preferring ligands with increased stability to enzymatic degradation and penetration to brain. Furthermore, these ligands exhibit potent antidepressant effect at notably low doses in several common rodent screening models of depression-like behavior.

In conclusion, this thesis provides a number of different novel galanin receptors binding ligands. Simultaneously, it demonstrates multiple different design strategies and chemical modifications that increase the binding and signaling properties, decrease susceptibility towards enzymatic degradation, and improve penetration to the brain. Additionally, this study provides a stable GalR3 cell line, a novel tool improving the possibilities for the characterization of galanin analogues. Furthermore, the results of this thesis have led to the hypothesis that GalR2 agonists could potentially be used as a treatment for mood disorders, and GalR1 antagonists might yield as drugs for acute suppression of appetite. However, in both cases further studies using more relevant and complex animal models need to be carried out.

Even though numerous peptide based ligands for galanin receptors have been produced during the last years, the field still lacks highly subtype specific ligands that could be administered systemically. As galanin is known to regulate numerous pathological and physiological processes it has become an obvious drug target. Thus, the validation of galanin receptors as drug targets is of great interest. So far, the use of transgenic animals and modern molecular biology has led the field in delineating the involvement of different receptors in various diseases. However, the caveat of using KO animals is the tendency of organisms to result in neurochemical compensatory mechanisms when lifelong genetic alterations are being made. Therefore, highly subtype specific and systemically active galanin receptor ligands would be extremely helpful in mapping the galaninergic system, and could potentially be used as treatment for several different diseases. Within this thesis we presented a series of systemically active peptides. Nevertheless, there is still a big obstacle to overcome, since even minor changes in the sequence of the peptide tend to decrease its binding properties and subtype specificity. Hopefully, in parallel with the continuous development of novel ligands, research will provide the field with X-ray structures for galanin receptors or galanin receptor-ligand complexes. This would rapidly advance the field and make the development of novel ligands easier by applying structure based ligand design.

6. SUMMARY IN ESTONIAN

Uudsed GalR2 spetsiifilised ligandid: nende mõju toitumisele ning depressiooni-laadsele käitumisele

Galaniin on 29 aminohappe pikkune neuropeptiid, mis avastati ligi 30 aastat tagasi Stockholmis, Karolinska Instituudis Viktor Muti ja tema kolleegide poolt. Galaniin reguleerib kolme spetsiifilise G-valguga seotud retseptori (GPCR) alatüüpide (GalR1-GalR3) interaktsioonide abil mitmeid bioloogilisi funktsioone nagu depressioon, ärevus, alkoholisõltuvus, ainevahetuse häired ja valu. Kõik kolm retseptori alatüüpi on galaniini suhtes kõrge afiinsusega, omades seejuures erinevaid funktsionaalseid omadusi ja signaaliülekande radasid. Seetõttu on neil täita erinevad ülesanded mitmesuguste haiguste ja patoloogiliste seisundite reguleerimisel, muutes galaniini ja tema spetsiifilised retseptorid olulisteks farmakoloogia märklaudadeks ning uurimisobjektideks neuroteaduses. Hoolimata sellest on retseptorite alatüüpide seotus erinevate haigustega senini täielikult määratlemata, mis on suurel määral põhjustatud galaniini retseptorite alatüüp-spetsiifiliste ligandide puuduse tõttu.

Käesoleva doktoritöö peamiseks eesmärgiks on galaniini alatüübile 2 (GalR2) spetsiifiliste ligandide disainimine, süntees ja omaduste uurimine nii *in vitro* kui ka *in vivo* tingimustes.

Täpsemalt, publikatsioonis I kirjeldatakse uudse GalR2 ligandi M1145 disaini, määratakse ligandi afiinsus galaniini membraansete retseptorite suhtes ja näidatakse M1145 võimet aktiveerida $G\alpha_{q/11}$-valgu poolt reguleeritavat signaaliülekande rada. Lisaks demonstreeritakse uudset rakuliini, mis toodab GalR3 ja on abiks uute ligandide omaduste iseloomustamiseks. Publikatsioonides II ja III sünteesiti, sarnaselt esimesele, rida galaniini analooge ja määrati nende afiinsus erinevate galaniini retseptorite alatüüpide suhtes. Lisaks kasutati loodud ligande GalR2 aktiveerimise mõju demonstreerimiseks toitumisele ning depressiooni-laadsele käitumisele. Publikatsioonis IV kirjeldatakse meetodit, kuidas suurendada peptiidsete ligandide stabiilsust ensümaatilise lagundamise suhtes ning võimet läbida aju-vere barjääri. Rakendades kirjeldatud metoodikat, sünteesiti rida uudseid galaniini analooge ning demonstreeriti seejuures nende võimet vähendada depressiooni-laadset käitumist erinevates loommudelites.

Kokkuvõtvalt, käesolevas doktoritöös esitletakse mitmeid strateegiaid uudsete ja samas süsteemselt toimivate GalR2 selektiivsete peptiidsete ligandide disainiks. Uudseid ühendeid on tulevikus võimalik kasutada erinevate galaniini retseptorite alatüüpide rolli määramiseks mitmesugustes haigustes. Lisaks uuritakse töös GalR2 seost depressiooni-laadse käitumise ning toitumisega, demonstreerides seejuures mõnede ligandide võimet vähendada depressiooni-laadset käitumist, sarnaselt laialdaselt kliinilises kasutuses oleva antidepressandi imipramiiniga.

7. ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my supervisors Ülo Langel and Jaak Järv for their continuous support throughout my studies. I particularly would like to thank Ülo for the opportunity to do my PhD in his group. It has been a great pleasure to work under your supervision, your immense knowledge of science has led the way in guiding my research. I would also like to thank Jaak for taking me into his lab at very early stages of my studies. Our talks have helped me make important decision in science as well as in life.

An important denominator throughout my PhD studies has been the galanin squad comprising Johan, Kaido, Kristin, Kent, and Jaanus. Working with you guys has been a joy.

Thank you Professor John K. Robinson and your group (Jimmy and Maria) for hosting me at Stony Brook University, thanks to your guidance and supervision inside and outside the lab my stay in New York was an educational adventure.

Bruno Conti, I spent the best days of my life in California. Thank you for taking me in and teaching me the ways of molecular biology as well as the way of life in CA. Your excitement towards science was a great inspiration to me.

The days in the lab wouldn't have been such a joy without cheerful Cecilia. Big thanks to Manuel, Marcia, Ed, and Nikki for making my stay in CA so pleasant.

I would like to thank my co-workers in Tartu: Imre Mäger, Taavi Lehto, Tõnis Lehto, Elo Eriste, Dana Copolovici, Julia Sohourušenko, Piret Arukuusk, Katrin Zirk, Katrin Krõlov, Nikita Oskolkov, Katja Frolova, and all the students.

I want to thank my former co-workers at Stockholm University: Per Lundin, Staffan Lindberg, Mats Hansen, Peter Guterstam, Andrés Muñoz-Alarcón, Kariem Ezzat, Mattias Hällbrink, Marie-Louise Tjörnhammer, Rannar Sillard, Maarja Mäe, Katri Rosenthal-Aizman, Henrik Johansson, Henrik Helmfors, Peter Järver, and Samir El Andaloussi.

Suured tänud kõigile toeks olnud lähedastele inimestele!

8. REFERENCES

Abramov, U., Floren, A., Echevarria, D. J., Brewer, A., Manuzon, H., Robinson, J. K., Bartfai, T., Vasar, E. and Langel, Ü. (2004) Regulation of feeding by galnon. *Neuropeptides,* **38,** 55–61.

Adams, A. C., Clapham, J. C., Wynick, D. and Speakman, J. R. (2008) Feeding behaviour in galanin knockout mice supports a role of galanin in fat intake and preference. *Journal of Neuroendocrinology,* **20,** 199–206.

Ahmad, S., O'Donnell, D., Payza, K. et al. (1998) Cloning and evaluation of the role of rat GALR-2, a novel subtype of galanin receptor, in the control of pain perception. *Annals of the New York Academy of Sciences,* **863,** 108–119.

Akabayashi, A., Koenig, J. I., Watanabe, Y., Alexander, J. T. and Leibowitz, S. F. (1994) Galanin-containing neurons in the paraventricular nucleus: a neurochemical marker for fat ingestion and body weight gain. *Proceedings of the National Academy of Sciences of the United States of America,* **91,** 10375–10379.

Andell-Jonsson, S., Xu, I. S., Bartfai, T., Xu, X. J. and Wiesenfeld-Hallin, Z. (1997) The effect of naturally occurring fragments of galanin message-associated peptide on spinal cord excitability in rats. *Neurosci Lett,* **235,** 154–156.

Anderson, M. E., Runesson, J., Saar, I., Langel, Ü. and Robinson, J. K. (2012) Galanin, through GalR1 but not GalR2 receptors, decreases motivation at times of high appetitive behavior. *Behavioural Brain Research,* **239C,** 90–93.

Anglade, I., Wang, Y., Jensen, J., Tramu, G., Kah, O. and Conlon, J. M. (1994) Characterization of trout galanin and its distribution in trout brain and pituitary. *The Journal of Comparative Neurology,* **350,** 63–74.

Association, A. P. (2000) Diagnostic and Statistical Manual of Mental Disorders. Washington, DC: American Psychiatric Association.

Badie-Mahdavi, H., Lu, X., Behrens, M. M. and Bartfai, T. (2005) Role of galanin receptor 1 and galanin receptor 2 activation in synaptic plasticity associated with 3',5'-cyclic AMP response element-binding protein phosphorylation in the dentate gyrus: studies with a galanin receptor 2 agonist and galanin receptor 1 knockout mice. *Neuroscience,* **133,** 591–604.

Barr, A. M., Kinney, J. W., Hill, M. N., Lu, X., Biros, S., Rebek, J., Jr. and Bartfai, T. (2006) A novel, systemically active, selective galanin receptor type-3 ligand exhibits antidepressant-like activity in preclinical tests. *Neurosci Lett,* **405,** 111–115.

Bartfai, T., Bedecs, K., Land, T. et al. (1991) M-15: high-affinity chimeric peptide that blocks the neuronal actions of galanin in the hippocampus, locus coeruleus, and spinal cord. *Proceedings of the National Academy of Sciences of the United States of America,* **88,** 10961–10965.

Bartfai, T., Langel, Ü., Bedecs, K. et al. (1993) Galanin-receptor ligand M40 peptide distinguishes between putative galanin-receptor subtypes. *Proceedings of the National Academy of Sciences of the United States of America,* **90,** 11287–11291.

Bartfai, T., Lu, X., Badie-Mahdavi, H. et al. (2004) Galmic, a nonpeptide galanin receptor agonist, affects behaviors in seizure, pain, and forced-swim tests. *Proceedings of the National Academy of Sciences of the United States of America,* **101,** 10470–10475.

Barton, C., Lin, L., York, D. A. and Bray, G. A. (1995) Differential effects of enterostatin, galanin and opioids on high-fat diet consumption. *Brain Research,* **702,** 55–60.

Bedecs, K., Langel, Ü. and Bartfai, T. (1995) Metabolism of galanin and galanin (1–16) in isolated cerebrospinal fluid and spinal cord membranes from rat. *Neuropeptides,* **29,** 137–143.

Berger, A., Lang, R., Moritz, K., Santic, R., Hermann, A., Sperl, W. and Kofler, B. (2004) Galanin receptor subtype GalR2 mediates apoptosis in SH-SY5Y neuroblastoma cells. *Endocrinology,* **145,** 500–507.

Borowsky, B., Walker, M. W., Huang, L. Y., Jones, K. A., Smith, K. E., Bard, J., Branchek, T. A. and Gerald, C. (1998) Cloning and characterization of the human galanin GALR2 receptor. *Peptides,* **19,** 1771–1781.

Boughton, C. K., Patterson, M., Bewick, G. A. et al. (2010) Alarin stimulates food intake and gonadotrophin release in male rats. *British Journal of Pharmacology,* **161,** 601–613.

Boyle, M. R., Verchere, C. B., McKnight, G., Mathews, S., Walker, K. and Taborsky, G. J., Jr. (1994) Canine galanin: sequence, expression and pancreatic effects. *Regulatory Peptides,* **50,** 1–11.

Branchek, T. A., Smith, K. E., Gerald, C. and Walker, M. W. (2000) Galanin receptor subtypes. *Trends in Pharmacological Sciences,* **21,** 109–117.

Bulaj, G., Green, B. R., Lee, H. K. et al. (2008) Design, synthesis, and characterization of high-affinity, systemically-active galanin analogues with potent anticonvulsant activities. *Journal of Medicinal Chemistry,* **51,** 8038–8047.

Ceide, S. C., Trembleau, L., Haberhauer, G., Somogyi, L., Lu, X., Bartfai, T. and Rebek, J., Jr. (2004) Synthesis of galmic: a nonpeptide galanin receptor agonist. *Proceedings of the National Academy of Sciences of the United States of America,* **101,** 16727–16732.

Chartrel, N., Wang, Y., Fournier, A., Vaudry, H. and Conlon, J. M. (1995) Frog vasoactive intestinal polypeptide and galanin: primary structures and effects on pituitary adenylate cyclase. *Endocrinology,* **136,** 3079–3086.

Cheung, C. C., Hohmann, J. G., Clifton, D. K. and Steiner, R. A. (2001) Distribution of galanin messenger RNA-expressing cells in murine brain and their regulation by leptin in regions of the hypothalamus. *Neuroscience,* **103,** 423–432.

Chu, M., Mierzwa, R., Truumees, I. et al. (1997) A new fungal metabolite, Sch 202596, with inhibitory activity in the galanin receptor GALR1 assay. *Tetrahedron Letters,* **38,** 6111–6114.

Church, W. B., Jones, K. A., Kuiper, D. A., Shine, J. and Iismaa, T. P. (2002) Molecular modelling and site-directed mutagenesis of human GALR1 galanin receptor defines determinants of receptor subtype specificity. *Protein Eng,* **15,** 313–323.

Clark, L., Chamberlain, S. R. and Sahakian, B. J. (2009) Neurocognitive mechanisms in depression: implications for treatment. *Annu Rev Neurosci,* **32,** 57–74.

Corwin, R. L., Robinson, J. K. and Crawley, J. N. (1993) Galanin antagonists block galanin-induced feeding in the hypothalamus and amygdala of the rat. *The European Journal of Neuroscience,* **5,** 1528–1533.

Crawley, J. N., Austin, M. C., Fiske, S. M., Martin, B., Consolo, S., Berthold, M., Langel, Ü., Fisone, G. and Bartfai, T. (1990) Activity of centrally administered galanin fragments on stimulation of feeding behavior and on galanin receptor binding in the rat hypothalamus. *J Neurosci,* **10,** 3695–3700.

Crawley, J. N., Robinson, J. K., Langel, Ü. and Bartfai, T. (1993) Galanin receptor antagonists M40 and C7 block galanin-induced feeding. *Brain Research,* **600,** 268–272.

Crisafulli, C., Fabbri, C., Porcelli, S., Drago, A., Spina, E., De Ronchi, D. and Serretti, A. (2011) Pharmacogenetics of antidepressants. *Frontiers in Pharmacology,* **2,** 6.

Cunningham, M. J., Scarlett, J. M. and Steiner, R. A. (2002) Cloning and distribution of galanin-like peptide mRNA in the hypothalamus and pituitary of the macaque. *Endocrinology,* **143,** 755–763.

Dasgupta, P., Singh, A. and Mukherjee, R. (2002) N-terminal acylation of somatostatin analog with long chain fatty acids enhances its stability and anti-proliferative activity in human breast adenocarcinoma cells. *Biological & Pharmaceutical Bulletin,* **25,** 29–36.

de Pedro, N., Cespedes, M. V., Delgado, M. J. and Alonso-Bedate, M. (1995) The galanin-induced feeding stimulation is mediated via alpha 2-adrenergic receptors in goldfish. *Regulatory Peptides,* **57,** 77–84.

Ding, X., MacTavish, D., Kar, S. and Jhamandas, J. H. (2006) Galanin attenuates beta-amyloid (Abeta) toxicity in rat cholinergic basal forebrain neurons. *Neurobiology of Disease,* **21,** 413–420.

Dube, M. G., Horvath, T. L., Leranth, C., Kalra, P. S. and Kalra, S. P. (1994) Naloxone reduces the feeding evoked by intracerebroventricular galanin injection. *Physiology & Behavior,* **56,** 811–813.

Eberhard, N., Mayer, C., Santic, R., Navio, R. P., Wagner, A., Bauer, H. C., Sperk, G., Boehm, U. and Kofler, B. (2012) Distribution of alarin immunoreactivity in the mouse brain. *J Mol Neurosci,* **46,** 18–32.

Eriksson, T. M., Delagrange, P., Spedding, M., Popoli, M., Mathe, A. A., Ögren, S. O. and Svenningsson, P. (2011) Emotional memory impairments in a genetic rat model of depression: involvement of 5-HT/MEK/Arc signaling in restoration. *Mol Psychiatry*.

Evans, H., Baumgartner, M., Shine, J. and Herzog, H. (1993) Genomic organization and localization of the gene encoding human preprogalanin. *Genomics,* **18,** 473–477.

Evans, H. F. and Shine, J. (1991) Human galanin: molecular cloning reveals a unique structure. *Endocrinology,* **129,** 1682–1684.

Fraley, G. S., Leathley, E., Lundy, N., Chheng, E., King, I. and Kofler, B. (2012a) Effects of alarin on food intake, body weight and luteinizing hormone secretion in male mice. *Neuropeptides,* **46,** 99–104.

Fraley, G. S., Leathley, E., Nickols, A., Gerometta, E., Coombs, E., Colton, S., Gallemore, S., Lindberg, A. and Kofler, B. (2012b) Alarin 6-25Cys antagonizes alarin-specific effects on food intake and luteinizing hormone secretion. *Neuropeptides*.

Gottsch, M. L., Zeng, H., Hohmann, J. G., Weinshenker, D., Clifton, D. K. and Steiner, R. A. (2005) Phenotypic analysis of mice deficient in the type 2 galanin receptor (GALR2). *Molecular and Cellular Biology,* **25,** 4804–4811.

Habert-Ortoli, E., Amiranoff, B., Loquet, I., Laburthe, M. and Mayaux, J. F. (1994) Molecular cloning of a functional human galanin receptor. *Proceedings of the National Academy of Sciences of the United States of America,* **91,** 9780–9783.

Habu, A., Ohishi, T., Mihara, S., Ohkubo, R., Hong, Y. M., Mochizuki, T. and Yanaihara, N. (1994) Isolation and Sequence Determination of Galanin from the Pituitary of Yellowfin Tuna. *Biomedical Research-Tokyo,* **15,** 357–362.

Hao, J. X., Shi, T. J., Xu, I. S., Kaupilla, T., Xu, X. J., Hökfelt, T., Bartfai, T. and Wiesenfeld-Hallin, Z. (1999) Intrathecal galanin alleviates allodynia-like behaviour in rats after partial peripheral nerve injury. *The European Journal of Neuroscience,* **11,** 427–432.

Heninger, G. R., Delgado, P. L. and Charney, D. S. (1996) The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. *Pharmacopsychiatry,* **29,** 2–11.
Hillebrand, J. J., de Wied, D. and Adan, R. A. (2002) Neuropeptides, food intake and body weight regulation: a hypothalamic focus. *Peptides,* **23,** 2283–2306.
Hohmann, J. G., Krasnow, S. M., Teklemichael, D. N., Clifton, D. K., Wynick, D. and Steiner, R. A. (2003) Neuroendocrine profiles in galanin-overexpressing and knockout mice. *Neuroendocrinology,* **77,** 354–366.
Holmes, A., Heilig, M., Rupniak, N. M., Steckler, T. and Griebel, G. (2003a) Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. *Trends in Pharmacological Sciences,* **24,** 580–588.
Holmes, A., Kinney, J. W., Wrenn, C. C. et al. (2003b) Galanin GAL-R1 receptor null mutant mice display increased anxiety-like behavior specific to the elevated plus-maze. *Neuropsychopharmacology,* **28,** 1031–1044.
Holub, B. S., Rauch, I., Radner, S., Sperl, W., Hell, M. and Kofler, B. (2011) Effects of galanin message-associated peptide and neuropeptide Y against various non-albicans Candida strains. *International Journal of Antimicrobial Agents,* **38,** 76–80.
Howard, A. D., Tan, C., Shiao, L. L. et al. (1997) Molecular cloning and characterization of a new receptor for galanin. *FEBS Letters,* **405,** 285–290.
Hökfelt, T., Aman, K., Arvidsson, U. et al. (1992) Galanin message-associated peptide (GMAP)- and galanin-like immunoreactivities: overlapping and differential distributions in the rat. *Neurosci Lett,* **142,** 139–142.
Jacoby, A. S., Webb, G. C., Liu, M. L., Kofler, B., Hort, Y. J., Fathi, Z., Bottema, C. D., Shine, J. and Iismaa, T. P. (1997) Structural organization of the mouse and human GALR1 galanin receptor genes (Galnr and GALNR) and chromosomal localization of the mouse gene. *Genomics,* **45,** 496–508.
Juréus, A., Cunningham, M. J., Li, D., Johnson, L. L., Krasnow, S. M., Teklemichael, D. N., Clifton, D. K. and Steiner, R. A. (2001) Distribution and regulation of galanin-like peptide (GALP) in the hypothalamus of the mouse. *Endocrinology,* **142,** 5140–5144.
Juréus, A., Cunningham, M. J., McClain, M. E., Clifton, D. K. and Steiner, R. A. (2000) Galanin-like peptide (GALP) is a target for regulation by leptin in the hypothalamus of the rat. *Endocrinology,* **141,** 2703–2706.
Jurkowski, W., Yazdi, S. and Elofsson, A. (2013) Ligand binding properties of human galanin receptors. *Molecular Membrane Biology*.
Kageyama, H., Kita, T., Toshinai, K. et al. (2006) Galanin-like peptide promotes feeding behaviour via activation of orexinergic neurones in the rat lateral hypothalamus. *Journal of Neuroendocrinology,* **18,** 33–41.
Kakuyama, H., Kuwahara, A., Mochizuki, T., Hoshino, M. and Yanaihara, N. (1997) Role of N-terminal active sites of galanin in neurally evoked circular muscle contractions in the guinea-pig ileum. *European journal of pharmacology,* **329,** 85–91.
Kaplan, L. M., Gabriel, S. M., Koenig, J. I., Sunday, M. E., Spindel, E. R., Martin, J. B. and Chin, W. W. (1988a) Galanin is an estrogen-inducible, secretory product of the rat anterior pituitary. *Proceedings of the National Academy of Sciences of the United States of America,* **85,** 7408–7412.
Kaplan, L. M., Spindel, E. R., Isselbacher, K. J. and Chin, W. W. (1988b) Tissue-specific expression of the rat galanin gene. *Proceedings of the National Academy of Sciences of the United States of America,* **85,** 1065–1069.

Karatayev, O., Baylan, J. and Leibowitz, S. F. (2009) Increased intake of ethanol and dietary fat in galanin overexpressing mice. *Alcohol (Fayetteville, N.Y,* **43,** 571–580.
Karatayev, O., Baylan, J., Weed, V., Chang, S., Wynick, D. and Leibowitz, S. F. (2010) Galanin knockout mice show disturbances in ethanol consumption and expression of hypothalamic peptides that stimulate ethanol intake. *Alcoholism, Clinical and Experimental Research,* **34,** 72–80.
Kask, K., Berthold, M., Bourne, J., Andell, S., Langel, Ü. and Bartfai, T. (1995) Binding and agonist/antagonist actions of M35, galanin(1–13)-bradykinin(2-9)amide chimeric peptide, in Rin m 5F insulinoma cells. *Regulatory Peptides,* **59,** 341–348.
Kask, K., Berthold, M., Kahl, U., Juréus, A., Nordvall, G., Langel, Ü. and Bartfai, T. (1998) Mutagenesis study on human galanin receptor GalR1 reveals domains involved in ligand binding. *Annals of the New York Academy of Sciences,* **863,** 78–85.
Kessler, R. C., Akiskal, H. S., Ames, M. et al. (2006) Prevalence and effects of mood disorders on work performance in a nationally representative sample of U.S. workers. *The American Journal of Psychiatry,* **163,** 1561–1568.
Kolakowski, L. F., Jr., O'Neill, G. P., Howard, A. D. et al. (1998) Molecular characterization and expression of cloned human galanin receptors GALR2 and GALR3. *Journal of Neurochemistry,* **71,** 2239–2251.
Konkel, M. J., Lagu, B., Boteju, L. W. et al. (2006a) 3-arylimino-2-indolones are potent and selective galanin GAL3 receptor antagonists. *Journal of Medicinal Chemistry,* **49,** 3757–3758.
Konkel, M. J., Packiarajan, M., Chen, H., Topiwala, U. P., Jimenez, H., Talisman, I. J., Coate, H. and Walker, M. W. (2006b) Amino substituted analogs of 1-phenyl-3-phenylimino-2-indolones with potent galanin Gal3 receptor binding affinity and improved solubility. *Bioorganic & Medicinal Chemistry Letters,* **16,** 3950–3954.
Krasnow, S. M., Hohmann, J. G., Gragerov, A., Clifton, D. K. and Steiner, R. A. (2004) Analysis of the contribution of galanin receptors 1 and 2 to the central actions of galanin-like peptide. *Neuroendocrinology,* **79,** 268–277.
Kuramochi, M., Onaka, T., Kohno, D., Kato, S. and Yada, T. (2006) Galanin-like peptide stimulates food intake via activation of neuropeptide Y neurons in the hypothalamic dorsomedial nucleus of the rat. *Endocrinology,* **147,** 1744–1752.
Kuteeva, E., Hökfelt, T., Wardi, T. and Ögren, S. O. (2008a) Galanin, galanin receptor subtypes and depression-like behaviour. *Cell Mol Life Sci,* **65,** 1854–1863.
Kuteeva, E., Wardi, T., Lundström, L., Sollenberg, U., Langel, Ü., Hökfelt, T. and Ögren, S. O. (2008b) Differential role of galanin receptors in the regulation of depression-like behavior and monoamine/stress-related genes at the cell body level. *Neuropsychopharmacology,* **33,** 2573–2585.
Kyrkouli, S. E., Stanley, B. G. and Leibowitz, S. F. (1986) Galanin: stimulation of feeding induced by medial hypothalamic injection of this novel peptide. *European journal of Pharmacology,* **122,** 159–160.
Kyrkouli, S. E., Stanley, B. G., Seirafi, R. D. and Leibowitz, S. F. (1990) Stimulation of feeding by galanin: anatomical localization and behavioral specificity of this peptide's effects in the brain. *Peptides,* **11,** 995–1001.
Land, T., Langel, U. and Bartfai, T. (1991a) Hypothalamic degradation of galanin(1–29) and galanin(1–16): identification and characterization of the peptidolytic products. *Brain Research,* **558,** 245–250.
Land, T., Langel, Ü., Low, M., Berthold, M., Unden, A. and Bartfai, T. (1991b) Linear and cyclic N-terminal galanin fragments and analogs as ligands at the hypothalamic

galanin receptor. *International Journal of Peptide and Protein Research,* **38,** 267–272.

Lang, R., Berger, A., Santic, R., Geisberger, R., Hermann, A., Herzog, H. and Kofler, B. (2005) Pharmacological and functional characterization of galanin-like peptide fragments as potent galanin receptor agonists. *Neuropeptides,* **39,** 179–184.

Lang, R., Gundlach, A. L. and Kofler, B. (2007) The galanin peptide family: receptor pharmacology, pleiotropic biological actions, and implications in health and disease. *Pharmacology & Therapeutics,* **115,** 177–207.

Leibowitz, S. F. (2005) Regulation and effects of hypothalamic galanin: relation to dietary fat, alcohol ingestion, circulating lipids and energy homeostasis. *Neuropeptides,* **39,** 327–332.

Leibowitz, S. F. and Kim, T. (1992) Impact of a galanin antagonist on exogenous galanin and natural patterns of fat ingestion. *Brain Research,* **599,** 148–152.

Liu, H. X., Brumovsky, P., Schmidt, R., Brown, W., Payza, K., Hodzic, L., Pou, C., Godbout, C. and Hökfelt, T. (2001) Receptor subtype-specific pronociceptive and analgesic actions of galanin in the spinal cord: selective actions via GalR1 and GalR2 receptors. *Proceedings of the National Academy of Sciences of the United States of America,* **98,** 9960–9964.

Lu, X., Barr, A. M., Kinney, J. W., Sanna, P., Conti, B., Behrens, M. M. and Bartfai, T. (2005a) A role for galanin in antidepressant actions with a focus on the dorsal raphe nucleus. *Proceedings of the National Academy of Sciences of the United States of America,* **102,** 874–879.

Lu, X., Lundström, L. and Bartfai, T. (2005b) Galanin (2–11) binds to GalR3 in transfected cell lines: limitations for pharmacological definition of receptor subtypes. *Neuropeptides,* **39,** 165–167.

Lu, X., Lundström, L., Langel, Ü. and Bartfai, T. (2005c) Galanin receptor ligands. *Neuropeptides,* **39,** 143–146.

Lu, X., Roberts, E., Xia, F. et al. (2010) GalR2-positive allosteric modulator exhibits anticonvulsant effects in animal models. *Proceedings of the National Academy of Sciences of the United States of America,* **107,** 15229–15234.

Lundkvist, J., Land, T., Kahl, U., Bedecs, K. and Bartfai, T. (1995) cDNA sequence, ligand biding, and regulation of galanin/GMAP in mouse brain. *Neurosci Lett,* **200,** 121–124.

Lundström, L., Sollenberg, U., Brewer, A. et al. (2005) A Galanin Receptor Subtype 1 Specific Agonist. *Int J Pept Res Ther,* **11,** 17–27.

Lundström, L., Sollenberg, U. E., Bartfai, T. and Langel, Ü. (2007) Molecular characterization of the ligand binding site of the human galanin receptor type 2, identifying subtype selective interactions. *Journal of Neurochemistry,* **103,** 1774–1784.

Ma, X., Tong, Y. G., Schmidt, R. et al. (2001) Effects of galanin receptor agonists on locus coeruleus neurons. *Brain Research,* **919,** 169–174.

Man, P. S. and Lawrence, C. B. (2008) Interleukin-1 mediates the anorexic and febrile actions of galanin-like Peptide. *Endocrinology,* **149,** 5791–5802.

Mathers, C. D., Vos, E. T., Stevenson, C. E. and Begg, S. J. (2000) The Australian Burden of Disease Study: measuring the loss of health from diseases, injuries and risk factors. *The Medical journal of Australia,* **172,** 592–596.

Melander, T., Hokfelt, T., Rokaeus, A., Cuello, A. C., Oertel, W. H., Verhofstad, A. and Goldstein, M. (1986) Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. *J Neurosci,* **6,** 3640–3654.

Mennicken, F., Hoffert, C., Pelletier, M., Ahmad, S. and O'Donnell, D. (2002) Restricted distribution of galanin receptor 3 (GalR3) mRNA in the adult rat central nervous system. *Journal of Chemical Neuroanatomy,* **24,** 257–268.

Merchenthaler, I., Lopez, F. J. and Negro-Vilar, A. (1993) Anatomy and physiology of central galanin-containing pathways. *Progress in Neurobiology,* **40,** 711–769.

Merrifield, R. B. (1963) Solid Phase Peptide Synthesis. 1. The synthesis of a tetra-peptide. . *J Am Chem Soc,* **85,** 2149–2154.

Miller, M. A., Kolb, P. E., Planas, B. and Raskind, M. A. (1998) Few cholinergic neurons in the rat basal forebrain coexpress galanin messenger RNA. *The Journal of Comparative Neurology,* **391,** 248–258.

Montgomery, S. A. (2006) Why do we need new and better antidepressants? *Int Clin Psychopharmacol,* **21 Suppl 1,** S1–S10.

Norberg, A., Sillard, R., Carlquist, M., Jornvall, H. and Mutt, V. (1991) Chemical detection of natural peptides by specific structures. Isolation of chicken galanin by monitoring for its N-terminal dipeptide, and determination of the amino acid sequence. *FEBS letters,* **288,** 151–153.

Ohman, A., Lycksell, P. O., Juréus, A., Langel, Ü., Bartfai, T. and Graslund, A. (1998) NMR study of the conformation and localization of porcine galanin in SDS micelles. Comparison with an inactive analog and a galanin receptor antagonist. *Biochemistry,* **37,** 9169–9178.

Ohtaki, T., Kumano, S., Ishibashi, Y. et al. (1999) Isolation and cDNA cloning of a novel galanin-like peptide (GALP) from porcine hypothalamus. *The Journal of Biological Chemistry,* **274,** 37041–37045.

Pieribone, V. A., Xu, Z. Q., Zhang, X., Grillner, S., Bartfai, T. and Hökfelt, T. (1995) Galanin induces a hyperpolarization of norepinephrine-containing locus coeruleus neurons in the brainstem slice. *Neuroscience,* **64,** 861–874.

Pooga, M., Juréus, A., Razaei, K. et al. (1998) Novel galanin receptor ligands. *J Pept Res,* **51,** 65–74.

Porsolt, R. D., Le Pichon, M. and Jalfre, M. (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature,* **266,** 730–732.

Rada, P., Avena, N. M., Leibowitz, S. F. and Hoebel, B. G. (2004) Ethanol intake is increased by injection of galanin in the paraventricular nucleus and reduced by a galanin antagonist. *Alcohol (Fayetteville, N.Y,* **33,** 91–97.

Rajarao, S. J., Platt, B., Sukoff, S. J. et al. (2007) Anxiolytic-like activity of the non-selective galanin receptor agonist, galnon. *Neuropeptides,* **41,** 307–320.

Rauch, I., Lundstrom, L., Hell, M., Sperl, W. and Kofler, B. (2007) Galanin message-associated peptide suppresses growth and the budded-to-hyphal-form transition of Candida albicans. *Antimicrobial Agents and Chemotherapy,* **51,** 4167–4170.

Robertson, C. R., Scholl, E. A., Pruess, T. H., Green, B. R., White, H. S. and Bulaj, G. (2010) Engineering galanin analogues that discriminate between GalR1 and GalR2 receptor subtypes and exhibit anticonvulsant activity following systemic delivery. *Journal of Medicinal Chemistry,* **53,** 1871–1875.

Rodriguez-Puertas, R., Nilsson, S., Pascual, J., Pazos, A. and Hökfelt, T. (1997) 125I-galanin binding sites in Alzheimer's disease: increases in hippocampal subfields and a decrease in the caudate nucleus. *Journal of Neurochemistry,* **68,** 1106–1113.

Runesson, J., Saar, I., Lundström, L., Järv, J. and Langel, Ü. (2009) A novel GalR2-specific peptide agonist. *Neuropeptides,* **43,** 187–192.

Runesson, J., Sollenberg, U. E., Jurkowski, W., Yazdi, S., Eriksson, E. E., Elofsson, A. and Lange, U. (2010a) Determining receptor-ligand interaction of human galanin receptor type 3. *Neurochemistry International,* **57,** 804–811.

Runesson, J., Sollenberg, U. E., Jurkowski, W., Yazdi, S., Eriksson, E. E., Elofsson, A. and Langel, Ü. (2010b) Determining receptor-ligand interaction of human galanin receptor type 3. *Neurochem Int,* **57,** 804–811.

Rökaeus, Å. and Brownstein, M. J. (1986) Construction of a porcine adrenal medullary cDNA library and nucleotide sequence analysis of two clones encoding a galanin precursor. *Proceedings of the National Academy of Sciences of the United States of America,* **83,** 6287–6291.

Rökaeus, Å., Young, W. S., 3rd and Mezey, E. (1988) Galanin coexists with vasopressin in the normal rat hypothalamus and galanin's synthesis is increased in the Brattleboro (diabetes insipidus) rat. *Neurosci Lett,* **90,** 45–50.

Saar, I., Runesson, J., McNamara, I., Järv, J., Robinson, J. K. and Langel, Ü. (2011) Novel galanin receptor subtype specific ligands in feeding regulation. *Neurochem Int,* **58,** 714–720.

Saar, K., Mazarati, A. M., Mahlapuu, R. et al. (2002) Anticonvulsant activity of a non-peptide galanin receptor agonist. *Proceedings of the National Academy of Sciences of the United States of America,* **99,** 7136–7141.

Sagi, V. N., Liu, T., Lu, X., Bartfai, T. and Roberts, E. (2011) Synthesis and biological evaluation of novel pyrimidine derivatives as sub-micromolar affinity ligands of GalR2. *Bioorganic & Medicinal Chemistry Letters,* **21,** 7210–7215.

Sahu, A. (1998) Leptin decreases food intake induced by melanin-concentrating hormone (MCH), galanin (GAL) and neuropeptide Y (NPY) in the rat. *Endocrinology,* **139,** 4739–4742.

Santic, R., Fenninger, K., Graf, K. et al. (2006) Gangliocytes in neuroblastic tumors express alarin, a novel peptide derived by differential splicing of the galanin-like peptide gene. *J Mol Neurosci,* **29,** 145–152.

Santic, R., Schmidhuber, S. M., Lang, R., Rauch, I., Voglas, E., Eberhard, N., Bauer, J. W., Brain, S. D. and Kofler, B. (2007) Alarin is a vasoactive peptide. *Proceedings of the National Academy of Sciences of the United States of America,* **104,** 10217–10222.

Schildkraut, J. J. (1965) The catecholamine hypothesis of affective disorders: a review of supporting evidence. *The American Journal of Psychiatry,* **122,** 509–522.

Schmidt, W. E., Kratzin, H., Eckart, K. et al. (1991) Isolation and primary structure of pituitary human galanin, a 30-residue nonamidated neuropeptide. *Proceedings of the National Academy of Sciences of the United States of America,* **88,** 11435–11439.

Scott, M. K., Ross, T. M., Lee, D. H. et al. (2000) 2,3-Dihydro-dithiin and -dithiepine-1,1,4,4-tetroxides: small molecule non-peptide antagonists of the human galanin hGAL-1 receptor. *Bioorganic & Medicinal Chemistry,* **8,** 1383–1391.

Seligman, M. E. and Maier, S. F. (1967) Failure to escape traumatic shock. *Journal of Experimental Psychology,* **74,** 1–9.

Seth, A., Stanley, S., Dhillo, W., Murphy, K., Ghatei, M. and Bloom, S. (2003) Effects of galanin-like peptide on food intake and the hypothalamo-pituitary-thyroid axis. *Neuroendocrinology,* **77,** 125–131.

Sillard, R., Langel, Ü. and Jornvall, H. (1991) Isolation and characterization of galanin from sheep brain. *Peptides,* **12,** 855–859.

Smith, K. E., Forray, C., Walker, M. W., Jones, K. A., Tamm, J. A., Bard, J., Branchek, T. A., Linemeyer, D. L. and Gerald, C. (1997) Expression cloning of a rat hypotha-

lamic galanin receptor coupled to phosphoinositide turnover. *The Journal of biological chemistry,* **272,** 24612–24616.

Smith, K. E., Walker, M. W., Artymyshyn, R. et al. (1998) Cloned human and rat galanin GALR3 receptors. Pharmacology and activation of G-protein inwardly rectifying K+ channels. *The Journal of biological chemistry,* **273,** 23321–23326.

Sollenberg, U. E., Lundström, L., Bartfai, T. and Langel, Ü. (2006) M871 – A Novel Peptide Antagonist Selectively Recognizingthe Galanin Receptor Type 2. *Int J Peptide Prot Res,* **12,** 115–119.

Sollenberg, U. E., Runesson, J., Sillard, R. and Langel, Ü. (2010) Binding of Chimeric Peptides M617 and M871 to Galanin Receptor Type 3 Reveals Characteristics of Galanin Receptor-Ligand Interaction. *Int J Peptide Prot Res,* **16,** 17–22.

Steru, L., Chermat, R., Thierry, B. and Simon, P. (1985) The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology,* **85,** 367–370.

Swanson, C. J., Blackburn, T. P., Zhang, X. et al. (2005a) Anxiolytic- and antidepressant-like profiles of the galanin-3 receptor (Gal3) antagonists SNAP 37889 and SNAP 398299. *Proceedings of the National Academy of Sciences of the United States of America,* **102,** 17489–17494.

Swanson, C. J., Blackburn, T. P., Zhang, X. et al. (2005b) Anxiolytic- and antidepressant-like profiles of the galanin-3 receptor (Gal3) antagonists SNAP 37889 and SNAP 398299. *Proceedings of the National Academy of Sciences of the United States of America,* **102,** 17489–17494.

Zhang, X., Nicholas, A. P. and Hökfelt, T. (1995) Ultrastructural studies on peptides in the dorsal horn of the rat spinal cord – II. Co-existence of galanin with other peptides in local neurons. *Neuroscience,* **64,** 875–891.

Zhang, X., Xu, Z. O., Shi, T. J. et al. (1998) Regulation of expression of galanin and galanin receptors in dorsal root ganglia and spinal cord after axotomy and inflammation. *Annals of the New York Academy of Sciences,* **863,** 402–413.

Zorrilla, E. P., Brennan, M., Sabino, V., Lu, X. and Bartfai, T. (2007) Galanin type 1 receptor knockout mice show altered responses to high-fat diet and glucose challenge. *Physiology & Behavior,* **91,** 479–485.

Tachibana, T., Mori, M., Khan, M. S., Ueda, H., Sugahara, K. and Hiramatsu, K. (2008) Central administration of galanin stimulates feeding behavior in chicks. *Comparative Biochemistry and Physiology,* **151,** 637–640.

Takatsu, Y., Matsumoto, H., Ohtaki, T., Kumano, S., Kitada, C., Onda, H., Nishimura, O. and Fujino, M. (2001) Distribution of galanin-like peptide in the rat brain. *Endocrinology,* **142,** 1626–1634.

Tatemoto, K., Rökaeus, Å., Jornvall, H., McDonald, T. J. and Mutt, V. (1983) Galanin – a novel biologically active peptide from porcine intestine. *FEBS letters,* **164,** 124–128.

Tempel, D. L., Leibowitz, K. J. and Leibowitz, S. F. (1988) Effects of PVN galanin on macronutrient selection. *Peptides,* **9,** 309–314.

Tritos, N. A. and Maratos-Flier, E. (1999) Two important systems in energy homeostasis: melanocortins and melanin-concentrating hormone. *Neuropeptides,* **33,** 339–349.

Tsutsui, K., Li, D., Ukena, K., Kikuchi, M. and Ishii, S. (1998) Developmental changes in galanin receptors in the quail oviduct and the effect of ovarian sex steroids on galanin receptor induction. *Endocrinology,* **139,** 4230–4236.

Van Der Kolk, N., Madison, F. N., Mohr, M., Eberhard, N., Kofler, B. and Fraley, G. S. (2010) Alarin stimulates food intake in male rats and LH secretion in castrated male rats. *Neuropeptides,* **44,** 333–340.

Wang, S., Hashemi, T., Fried, S., Clemmons, A. L. and Hawes, B. E. (1998) Differential intracellular signaling of the GalR1 and GalR2 galanin receptor subtypes. *Biochemistry,* **37,** 6711–6717.

Wang, S., Hashemi, T., He, C., Strader, C. and Bayne, M. (1997a) Molecular cloning and pharmacological characterization of a new galanin receptor subtype. *Molecular Pharmacology,* **52,** 337–343.

Wang, S., He, C., Hashemi, T. and Bayne, M. (1997b) Cloning and expressional characterization of a novel galanin receptor. Identification of different pharmacophores within galanin for the three galanin receptor subtypes. *The Journal of Biological Chemistry,* **272,** 31949–31952.

Wang, Y. and Conlon, J. M. (1994a) Purification and characterization of galanin from the phylogenetically ancient fish, the bowfin (Amia calva) and dogfish (Scyliorhinus canicula). *Peptides,* **15,** 981–986.

Wang, Y. and Conlon, J. M. (1994b) Purification and primary structure of galanin from the alligator stomach. *Peptides,* **15,** 603–606.

Wang, Y., Lance, V. A., Nielsen, P. F. and Conlon, J. M. (1999) Neuroendocrine peptides (insulin, pancreatic polypeptide, neuropeptide Y, galanin, somatostatin, substance P, and neuropeptide gamma) from the desert tortoise, Gopherus agassizii. *Peptides,* **20,** 713–722.

Waters, S. M. and Krause, J. E. (2000) Distribution of galanin-1, -2 and -3 receptor messenger RNAs in central and peripheral rat tissues. *Neuroscience,* **95,** 265–271.

Weiss, J. M., Goodman, P. A., Losito, B. G., Corrigan, S., Charry, J. M. and Bailey, W. H. (1981) Behavioral Depression Produced by an Uncontrollable Stressor – Relationship to Norepinephrine, Dopamine, and Serotonin Levels in Various Regions of Rat-Brain. *Brain Research Reviews,* **3,** 167–205.

WFMH (2010) Mental Health and Chronic Physical Illnesses. The need for continued and integrated care., Vol. 2012. World Federation for Mental Health, http://www.wfmh.org/2010DOCS/WMHDAY2010.pdf

White, H. S., Scholl, E. A., Klein, B. D., Flynn, S. P., Pruess, T. H., Green, B. R., Zhang, L. and Bulaj, G. (2009) Developing novel antiepileptic drugs: characterization of NAX 5055, a systemically-active galanin analog, in epilepsy models. *Neurotherapeutics: the journal of the American Society for Experimental NeuroTherapeutics,* **6,** 372–380.

Wiesenfeld-Hallin, Z., Xu, X. J., Crawley, J. N. and Hökfelt, T. (2005) Galanin and spinal nociceptive mechanisms: recent results from transgenic and knock-out models. *Neuropeptides,* **39,** 207–210.

Willner, P. (1997) Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology,* **134,** 319–329.

Witt, K. A. and Davis, T. P. (2006) CNS drug delivery: opioid peptides and the blood-brain barrier. *The AAPS journal,* **8,** E76–88.

Wittau, N., Grosse, R., Kalkbrenner, F., Gohla, A., Schultz, G. and Gudermann, T. (2000) The galanin receptor type 2 initiates multiple signaling pathways in small cell lung cancer cells by coupling to G(q), G(i) and G(12) proteins. *Oncogene,* **19,** 4199–4209.

Wrenn, C. C., Kinney, J. W., Marriott, L. K. et al. (2004) Learning and memory performance in mice lacking the GAL-R1 subtype of galanin receptor. *The European Journal of Neuroscience,* **19,** 1384–1396.

Wu, W. P., Hao, J. X., Lundström, L., Wiesenfeld-Hallin, Z., Langel, Ü., Bartfai, T. and Xu, X. J. (2003) Systemic galnon, a low-molecular weight galanin receptor agonist, reduces heat hyperalgesia in rats with nerve injury. *European Journal of Pharmacology,* **482,** 133–137.

Ögren, S. O., Hökfelt, T., Kask, K., Langel, Ü. and Bartfai, T. (1992) Evidence for a role of the neuropeptide galanin in spatial learning. *Neuroscience,* **51,** 1–5.

Xu, Z. Q., Zhang, X., Pieribone, V. A., Grillner, S. and Hökfelt, T. (1998) Galanin-5-hydroxytryptamine interactions: electrophysiological, immunohistochemical and in situ hybridization studies on rat dorsal raphe neurons with a note on galanin R1 and R2 receptors. *Neuroscience,* **87,** 79–94.

Xu, X. J., Andell, S., Hao, J. X., Wiesenfeld-Hallin, Z. and Bartfai, T. (1995a) The effects of intrathecal galanin message-associated peptide (GMAP) on the flexor reflex in rats. *Regulatory Peptides,* **58,** 19–24.

Xu, X. J., Andell, S., Zhang, X., Wiesenfeld-Hallin, Z., Langel, Ü., Bedecs, K., Hökfelt, T. and Bartfai, T. (1995b) Peripheral axotomy increases the expression of galanin message-associated peptide (GMAP) in dorsal root ganglion cells and alters the effects of intrathecal GMAP on the flexor reflex in the rat. *Neuropeptides,* **28,** 299–307.

Yun, R., Dourmashkin, J. T., Hill, J., Gayles, E. C., Fried, S. K. and Leibowitz, S. F. (2005) PVN galanin increases fat storage and promotes obesity by causing muscle to utilize carbohydrate more than fat. *Peptides,* **26,** 2265–2273.

PUBLICATIONS

CURRICULUM VITAE

Name: Indrek Saar
Date of birth: 02.02.1984
Citizenship: Estonian
Contact: Institute of Technology
Faculty of Science and Technology
University of Tartu
Nooruse 1, 50411 Tartu, Estonia
E-mail: indrek_saar@hotmail.com

Education and professional employment

1997–2003 Haapsalu Wiedemanni Gymnasium
2003–2004 Conscription in Estonian Defence Forces, Narva-Jõesuu Border Guard Training Centre
2004–2007 University of Tartu – Bachelor degree in chemistry
2007–2009 University of Tartu – Master`s degree in Organic Chemistry (*cum laude*)
2008– University of Tartu, Institute of Technology, specialist
2009– University of Tartu, Institute of Chemistry, PhD student

Special courses:

2011–2012 The Scripps Research Institute (USA), Department of Molecular and Integrative Neurosciences, Graduate Research Assistant
2010–2011 Stockholm University (Sweden), Department of Neurochemistry, Graduate Research Assistant
2010 Stony Brook University (USA), Graduate Research Assistant
2008–2009 Stockholm University (Sweden), Department of Neurochemistry, visiting Master`s student

List of Publications:

Saar, I., Runesson, J., McNamara, I., Järv, J., Robinson, J.K., and Langel, Ü. (2011) Novel galanin receptor subtype specific ligands in feeding regulation. Neurochemistry International, 58, 714–720

Runesson, J., Saar, I., Lundström, L., Järv, I., and Langel, Ü. (2009) A novel GalR2-specific peptide agonist. Neuropeptides 43, 187–192

Saar, I., Runesson, J., Järv, J., Kurrikoff. K, and Langel, Ü. Novel galanin receptor subtype specific ligand in depression like behavior. Neurochemical Research, (2013) 38:398–404

Morrison, B.E., Marcondes, M.C.G., Nomura, D.K., Sanchez-Alavez M., Sanchez-Gonzalez A., Saar, I., Bartfai, T., Maher, P., Sugama,S., Conti, B. IL-13Rα1 expression in dopaminergic neurons contributes to their loss following chronic systemic treatment with LPS. Journal of Immunology, 2012; 189:5498–5502

Anderson, M.E., Runesson, J., Saar, I., Langel, Ü., Robinson, J.K. The Role of Galanin Systems in Appetite and Reward, Behavioural Brain Research, 239 (2013) 90– 93

Runesson, J., Saar, I., Järv, J., Langel, Ü. (2009). Novel peptide agonists, favoring galanin receptor type 2 over galanin receptor type 1 and 3. In: FEBS JOURNAL: 34th Congress of the Federation-of-European-Biochemical-Societies; Prague, CZECH REPUBLIC; JUL 04-09, 2009. , 2009, (Suppl. 1)

Saar, I., Lahe, J., Langel, K., Runesson, J., Webling, K., Järv, J., Rytkönen, J., Närvanen, A., Kurrikoff. K., and Langel, Ü. Novel systemically active galanin receptor subtype specific ligands in depressioon-like behaviour. Journal of Neurochemistry, resubmitted.

Runesson, J., Groves-Chapman, J.L., Webling, K., Saar, I., Sillard, R., Holmes, P.V., and Langel, Ü. Pharmacological stimulation of Galanin receptor 1 but not Galanin receptor 2 attenuates kainic acid-induced neuronal cell death in the rat hippocampus. Neuropeptides, submitted.

ELULOOKIRJELDUS

Nimi: Indrek Saar
Sünniaeg: 02.02.1984
Kodakondsus: Eesti
Kontakt: Tehnoloogiainstituut
Loodus- ja tehnoloogiateaduskond
Tartu Ülikool
Nooruse 1, 50411 Tartu, Eesti
E-post: indrek_saar@hotmail.com

Haridus ja erialane teenistuskäik:

1997–2003	Haapsalu Wiedemanni Gümnaasium
2003–2004	Ajateenistus Eesti Kaitseväes, Narva-Jõesuu Piirivalve Õppekeskus
2004–2007	Tartu Ülikool – Bakalaureuse kraad keemias
2007–2009	Tartu Ülikool – Magistri kraad orgaanilises keemias (*cum laude*)
2008–	Tartu Ülikool, Tehnoloogiainstituut, spetsialist
2009–	Tartu Ülikool, Keemia instituut, doktorant

Erialane enesetäiendus:

2011–2012	Scripps'i Uurimisinstituut (USA), Molekulaarse ja Integratiivse Neuroteaduste instituut, teadur
2010–2011	Stockholmi Ülikool (Rootsi) Neurokeemia instituut, teadur
2010	Stony Brook Ülikool (USA), teadur
2008–2009	Stockholmi Ülikool (Rootsi), Neurokeemia instituut, külalis-üliõpilane (magistrant)

Teaduspublikatsioonid:

Saar, I., Runesson, J., McNamara, I., Järv, J., Robinson, J.K., and Langel, Ü. (2011) Novel galanin receptor subtype specific ligands in feeding regulation. Neurochemistry International, 58, 714–720

Runesson, J., Saar, I., Lundström, L., Järv, I., and Langel, Ü. (2009) A novel GalR2-specific peptide agonist. Neuropeptides 43, 187–192

Saar, I., Runesson, J., Järv, J., Kurrikoff. K, and Langel, Ü. Novel galanin receptor subtype specific ligand in depression like behavior. Neurochemical Research, (2013) 38:398–404

Morrison, B.E., Marcondes, M.C.G., Nomura, D.K., Sanchez-Alavez M., Sanchez-Gonzalez A., Saar, I., Bartfai' T., Maher, P., Sugama,S., Conti, B. IL-13Rα1 expression in dopaminergic neurons contributes to their loss following chronic systemic treatment with LPS. Journal of Immunology, 2012; 189:5498–5502

Anderson, M.E., Runesson, J., Saar, I., Langel, Ü., Robinson, J.K. The Role of Galanin Systems in Appetite and Reward, Behavioural Brain Research, 239 (2013) 90– 93

Runesson, J., Saar, I., Järv, J., Langel, Ü. (2009). Novel peptide agonists, favoring galanin receptor type 2 over galanin receptor type 1 and 3. In: FEBS JOURNAL: 34th Congress of the Federation-of-European-Biochemical-Societies; Prague, CZECH REPUBLIC; JUL 04-09, 2009. , 2009, (Suppl. 1)

Saar, I., Lahe, J., Langel, K., Runesson, J., Webling, K., Järv, J., Rytkönen, J., Närvanen, A., Kurrikoff. K., and Langel, Ü. Novel systemically active galanin receptor subtype specific ligands in depressioon-like behaviour. Journal of Neurochemistry, resubmitted.

Runesson, J., Groves-Chapman, J.L., Webling, K., Saar, I., Sillard, R., Holmes, P.V., and Langel, Ü. Pharmacological stimulation of Galanin receptor 1 but not Galanin receptor 2 attenuates kainic acid-induced neuronal cell death in the rat hippocampus. Neuropeptides, submitted.

DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS

1. **Toomas Tamm.** Quantum-chemical simulation of solvent effects. Tartu, 1993, 110 p.
2. **Peeter Burk.** Theoretical study of gas-phase acid-base equilibria. Tartu, 1994, 96 p.
3. **Victor Lobanov.** Quantitative structure-property relationships in large descriptor spaces. Tartu, 1995, 135 p.
4. **Vahur Mäemets.** The ^{17}O and ^{1}H nuclear magnetic resonance study of H_2O in individual solvents and its charged clusters in aqueous solutions of electrolytes. Tartu, 1997, 140 p.
5. **Andrus Metsala.** Microcanonical rate constant in nonequilibrium distribution of vibrational energy and in restricted intramolecular vibrational energy redistribution on the basis of slater's theory of unimolecular reactions. Tartu, 1997, 150 p.
6. **Uko Maran.** Quantum-mechanical study of potential energy surfaces in different environments. Tartu, 1997, 137 p.
7. **Alar Jänes.** Adsorption of organic compounds on antimony, bismuth and cadmium electrodes. Tartu, 1998, 219 p.
8. **Kaido Tammeveski.** Oxygen electroreduction on thin platinum films and the electrochemical detection of superoxide anion. Tartu, 1998, 139 p.
9. **Ivo Leito.** Studies of Brønsted acid-base equilibria in water and non-aqueous media. Tartu, 1998, 101 p.
10. **Jaan Leis.** Conformational dynamics and equilibria in amides. Tartu, 1998, 131 p.
11. **Toonika Rinken.** The modelling of amperometric biosensors based on oxidoreductases. Tartu, 2000, 108 p.
12. **Dmitri Panov.** Partially solvated Grignard reagents. Tartu, 2000, 64 p.
13. **Kaja Orupõld.** Treatment and analysis of phenolic wastewater with microorganisms. Tartu, 2000, 123 p.
14. **Jüri Ivask.** Ion Chromatographic determination of major anions and cations in polar ice core. Tartu, 2000, 85 p.
15. **Lauri Vares.** Stereoselective Synthesis of Tetrahydrofuran and Tetrahydropyran Derivatives by Use of Asymmetric Horner-Wadsworth-Emmons and Ring Closure Reactions. Tartu, 2000, 184 p.
16. **Martin Lepiku.** Kinetic aspects of dopamine D_2 receptor interactions with specific ligands. Tartu, 2000, 81 p.
17. **Katrin Sak.** Some aspects of ligand specificity of P2Y receptors. Tartu, 2000, 106 p.
18. **Vello Pällin.** The role of solvation in the formation of iotsitch complexes. Tartu, 2001, 95 p.

19. **Katrin Kollist.** Interactions between polycyclic aromatic compounds and humic substances. Tartu, 2001, 93 p.
20. **Ivar Koppel.** Quantum chemical study of acidity of strong and superstrong Brønsted acids. Tartu, 2001, 104 p.
21. **Viljar Pihl.** The study of the substituent and solvent effects on the acidity of OH and CH acids. Tartu, 2001, 132 p.
22. **Natalia Palm.** Specification of the minimum, sufficient and significant set of descriptors for general description of solvent effects. Tartu, 2001, 134 p.
23. **Sulev Sild.** QSPR/QSAR approaches for complex molecular systems. Tartu, 2001, 134 p.
24. **Ruslan Petrukhin.** Industrial applications of the quantitative structure-property relationships. Tartu, 2001, 162 p.
25. **Boris V. Rogovoy.** Synthesis of (benzotriazolyl)carboximidamides and their application in relations with *N*- and *S*-nucleophyles. Tartu, 2002, 84 p.
26. **Koit Herodes.** Solvent effects on UV-vis absorption spectra of some solvatochromic substances in binary solvent mixtures: the preferential solvation model. Tartu, 2002, 102 p.
27. **Anti Perkson.** Synthesis and characterisation of nanostructured carbon. Tartu, 2002, 152 p.
28. **Ivari Kaljurand.** Self-consistent acidity scales of neutral and cationic Brønsted acids in acetonitrile and tetrahydrofuran. Tartu, 2003, 108 p.
29. **Karmen Lust.** Adsorption of anions on bismuth single crystal electrodes. Tartu, 2003, 128 p.
30. **Mare Piirsalu.** Substituent, temperature and solvent effects on the alkaline hydrolysis of substituted phenyl and alkyl esters of benzoic acid. Tartu, 2003, 156 p.
31. **Meeri Sassian.** Reactions of partially solvated Grignard reagents. Tartu, 2003, 78 p.
32. **Tarmo Tamm.** Quantum chemical modelling of polypyrrole. Tartu, 2003. 100 p.
33. **Erik Teinemaa.** The environmental fate of the particulate matter and organic pollutants from an oil shale power plant. Tartu, 2003. 102 p.
34. **Jaana Tammiku-Taul.** Quantum chemical study of the properties of Grignard reagents. Tartu, 2003. 120 p.
35. **Andre Lomaka.** Biomedical applications of predictive computational chemistry. Tartu, 2003. 132 p.
36. **Kostyantyn Kirichenko.** Benzotriazole – Mediated Carbon–Carbon Bond Formation. Tartu, 2003. 132 p.
37. **Gunnar Nurk.** Adsorption kinetics of some organic compounds on bismuth single crystal electrodes. Tartu, 2003, 170 p.
38. **Mati Arulepp.** Electrochemical characteristics of porous carbon materials and electrical double layer capacitors. Tartu, 2003, 196 p.

39. **Dan Cornel Fara.** QSPR modeling of complexation and distribution of organic compounds. Tartu, 2004, 126 p.
40. **Riina Mahlapuu.** Signalling of galanin and amyloid precursor protein through adenylate cyclase. Tartu, 2004, 124 p.
41. **Mihkel Kerikmäe.** Some luminescent materials for dosimetric applications and physical research. Tartu, 2004, 143 p.
42. **Jaanus Kruusma.** Determination of some important trace metal ions in human blood. Tartu, 2004, 115 p.
43. **Urmas Johanson.** Investigations of the electrochemical properties of poly-pyrrole modified electrodes. Tartu, 2004, 91 p.
44. **Kaido Sillar.** Computational study of the acid sites in zeolite ZSM-5. Tartu, 2004, 80 p.
45. **Aldo Oras.** Kinetic aspects of dATPαS interaction with $P2Y_1$ receptor. Tartu, 2004, 75 p.
46. **Erik Mölder.** Measurement of the oxygen mass transfer through the air-water interface. Tartu, 2005, 73 p.
47. **Thomas Thomberg.** The kinetics of electroreduction of peroxodisulfate anion on cadmium (0001) single crystal electrode. Tartu, 2005, 95 p.
48. **Olavi Loog.** Aspects of condensations of carbonyl compounds and their imine analogues. Tartu, 2005, 83 p.
49. **Siim Salmar.** Effect of ultrasound on ester hydrolysis in aqueous ethanol. Tartu, 2006, 73 p.
50. **Ain Uustare.** Modulation of signal transduction of heptahelical receptors by other receptors and G proteins. Tartu, 2006, 121 p.
51. **Sergei Yurchenko.** Determination of some carcinogenic contaminants in food. Tartu, 2006, 143 p.
52. **Kaido Tämm.** QSPR modeling of some properties of organic compounds. Tartu, 2006, 67 p.
53. **Olga Tšubrik.** New methods in the synthesis of multisubstituted hydra-zines. Tartu. 2006, 183 p.
54. **Lilli Sooväli.** Spectrophotometric measurements and their uncertainty in chemical analysis and dissociation constant measurements. Tartu, 2006, 125 p.
55. **Eve Koort.** Uncertainty estimation of potentiometrically measured ph and pK_a values. Tartu, 2006, 139 p.
56. **Sergei Kopanchuk.** Regulation of ligand binding to melanocortin receptor subtypes. Tartu, 2006, 119 p.
57. **Silvar Kallip.** Surface structure of some bismuth and antimony single crystal electrodes. Tartu, 2006, 107 p.
58. **Kristjan Saal.** Surface silanization and its application in biomolecule coupling. Tartu, 2006, 77 p.
59. **Tanel Tätte.** High viscosity $Sn(OBu)_4$ oligomeric concentrates and their applications in technology. Tartu, 2006, 91 p.

60. **Dimitar Atanasov Dobchev**. Robust QSAR methods for the prediction of properties from molecular structure. Tartu, 2006, 118 p.
61. **Hannes Hagu**. Impact of ultrasound on hydrophobic interactions in solutions. Tartu, 2007, 81 p.
62. **Rutha Jäger.** Electroreduction of peroxodisulfate anion on bismuth electrodes. Tartu, 2007, 142 p.
63. **Kaido Viht.** Immobilizable bisubstrate-analogue inhibitors of basophilic protein kinases: development and application in biosensors. Tartu, 2007, 88 p.
64. **Eva-Ingrid Rõõm.** Acid-base equilibria in nonpolar media. Tartu, 2007, 156 p.
65. **Sven Tamp.** DFT study of the cesium cation containing complexes relevant to the cesium cation binding by the humic acids. Tartu, 2007, 102 p.
66. **Jaak Nerut.** Electroreduction of hexacyanoferrate(III) anion on Cadmium (0001) single crystal electrode. Tartu, 2007, 180 p.
67. **Lauri Jalukse.** Measurement uncertainty estimation in amperometric dissolved oxygen concentration measurement. Tartu, 2007, 112 p.
68. **Aime Lust.** Charge state of dopants and ordered clusters formation in CaF_2:Mn and CaF_2:Eu luminophors. Tartu, 2007, 100 p.
69. **Iiris Kahn**. Quantitative Structure-Activity Relationships of environmentally relevant properties. Tartu, 2007, 98 p.
70. **Mari Reinik.** Nitrates, nitrites, N-nitrosamines and polycyclic aromatic hydrocarbons in food: analytical methods, occurrence and dietary intake. Tartu, 2007, 172 p.
71. **Heili Kasuk.** Thermodynamic parameters and adsorption kinetics of organic compounds forming the compact adsorption layer at Bi single crystal electrodes. Tartu, 2007, 212 p.
72. **Erki Enkvist.** Synthesis of adenosine-peptide conjugates for biological applications. Tartu, 2007, 114 p.
73. **Svetoslav Hristov Slavov**. Biomedical applications of the QSAR approach. Tartu, 2007, 146 p.
74. **Eneli Härk.** Electroreduction of complex cations on electrochemically polished Bi(*hkl*) single crystal electrodes. Tartu, 2008, 158 p.
75. **Priit Möller.** Electrochemical characteristics of some cathodes for medium temperature solid oxide fuel cells, synthesized by solid state reaction technique. Tartu, 2008, 90 p.
76. **Signe Viggor.** Impact of biochemical parameters of genetically different pseudomonads at the degradation of phenolic compounds. Tartu, 2008, 122 p.
77. **Ave Sarapuu.** Electrochemical reduction of oxygen on quinone-modified carbon electrodes and on thin films of platinum and gold. Tartu, 2008, 134 p.
78. **Agnes Kütt.** Studies of acid-base equilibria in non-aqueous media. Tartu, 2008, 198 p.

79. **Rouvim Kadis.** Evaluation of measurement uncertainty in analytical chemistry: related concepts and some points of misinterpretation. Tartu, 2008, 118 p.
80. **Valter Reedo.** Elaboration of IVB group metal oxide structures and their possible applications. Tartu, 2008, 98 p.
81. **Aleksei Kuznetsov.** Allosteric effects in reactions catalyzed by the cAMP-dependent protein kinase catalytic subunit. Tartu, 2009, 133 p.
82. **Aleksei Bredihhin.** Use of mono- and polyanions in the synthesis of multisubstituted hydrazine derivatives. Tartu, 2009, 105 p.
83. **Anu Ploom.** Quantitative structure-reactivity analysis in organosilicon chemistry. Tartu, 2009, 99 p.
84. **Argo Vonk.** Determination of adenosine A_{2A}- and dopamine D_1 receptor-specific modulation of adenylate cyclase activity in rat striatum. Tartu, 2009, 129 p.
85. **Indrek Kivi.** Synthesis and electrochemical characterization of porous cathode materials for intermediate temperature solid oxide fuel cells. Tartu, 2009, 177 p.
86. **Jaanus Eskusson.** Synthesis and characterisation of diamond-like carbon thin films prepared by pulsed laser deposition method. Tartu, 2009, 117 p.
87. **Marko Lätt.** Carbide derived microporous carbon and electrical double layer capacitors. Tartu, 2009, 107 p.
88. **Vladimir Stepanov.** Slow conformational changes in dopamine transporter interaction with its ligands. Tartu, 2009, 103 p.
89. **Aleksander Trummal.** Computational Study of Structural and Solvent Effects on Acidities of Some Brønsted Acids. Tartu, 2009, 103 p.
90. **Eerold Vellemäe.** Applications of mischmetal in organic synthesis. Tartu, 2009, 93 p.
91. **Sven Parkel.** Ligand binding to 5-HT_{1A} receptors and its regulation by Mg^{2+} and Mn^{2+}. Tartu, 2010, 99 p.
92. **Signe Vahur.** Expanding the possibilities of ATR-FT-IR spectroscopy in determination of inorganic pigments. Tartu, 2010, 184 p.
93. **Tavo Romann**. Preparation and surface modification of bismuth thin film, porous, and microelectrodes. Tartu, 2010, 155 p.
94. **Nadežda Aleksejeva.** Electrocatalytic reduction of oxygen on carbon nanotube-based nanocomposite materials. Tartu, 2010, 147 p.
95. **Marko Kullapere.** Electrochemical properties of glassy carbon, nickel and gold electrodes modified with aryl groups. Tartu, 2010, 233 p.
96. **Liis Siinor.** Adsorption kinetics of ions at Bi single crystal planes from aqueous electrolyte solutions and room-temperature ionic liquids. Tartu, 2010, 101 p.
97. **Angela Vaasa.** Development of fluorescence-based kinetic and binding assays for characterization of protein kinases and their inhibitors. Tartu 2010, 101 p.

98. **Indrek Tulp.** Multivariate analysis of chemical and biological properties. Tartu 2010, 105 p.
99. **Aare Selberg.** Evaluation of environmental quality in Northern Estonia by the analysis of leachate. Tartu 2010, 117 p.
100. **Darja Lavõgina.** Development of protein kinase inhibitors based on adenosine analogue-oligoarginine conjugates. Tartu 2010, 248 p.
101. **Laura Herm.** Biochemistry of dopamine D_2 receptors and its association with motivated behaviour. Tartu 2010, 156 p.
102. **Terje Raudsepp.** Influence of dopant anions on the electrochemical properties of polypyrrole films. Tartu 2010, 112 p.
103. **Margus Marandi.** Electroformation of Polypyrrole Films: *In-situ* AFM and STM Study. Tartu 2011, 116 p.
104. **Kairi Kivirand.** Diamine oxidase-based biosensors: construction and working principles. Tartu, 2011, 140 p.
105. **Anneli Kruve.** Matrix effects in liquid-chromatography electrospray mass-spectrometry. Tartu, 2011, 156 p.
106. **Gary Urb.** Assessment of environmental impact of oil shale fly ash from PF and CFB combustion. Tartu, 2011, 108 p.
107. **Nikita Oskolkov.** A novel strategy for peptide-mediated cellular delivery and induction of endosomal escape. Tartu, 2011, 106 p.
108. **Dana Martin.** The QSPR/QSAR approach for the prediction of properties of fullerene derivatives. Tartu, 2011, 98 p.
109. **Säde Viirlaid.** Novel glutathione analogues and their antioxidant activity. Tartu, 2011, 106 p.
110. **Ülis Sõukand.** Simultaneous adsorption of Cd^{2+}, Ni^{2+}, and Pb^{2+} on peat. Tartu, 2011, 124 p.
111. **Lauri Lipping.** The acidity of strong and superstrong Brønsted acids, an outreach for the "limits of growth": a quantum chemical study. Tartu, 2011, 124 p.
112. **Heisi Kurig.** Electrical double-layer capacitors based on ionic liquids as electrolytes. Tartu, 2011, 146 p.
113. **Marje Kasari.** Bisubstrate luminescent probes, optical sensors and affinity adsorbents for measurement of active protein kinases in biological samples. Tartu, 2012, 126 p.
114. **Kalev Takkis.** Virtual screening of chemical databases for bioactive molecules. Tartu, 2012, 122 p.
115. **Ksenija Kisseljova**. Synthesis of aza-β^3-amino acid containing peptides and kinetic study of their phosphorylation by protein kinase A. Tartu, 2012, 104 p.
116. **Riin Rebane.** Advanced method development strategy for derivatization LC/ESI/MS. Tartu, 2012, 184 p.

117. **Vladislav Ivaništšev.** Double layer structure and adsorption kinetics of ions at metal electrodes in room temperature ionic liquids. Tartu, 2012, 128 p.
118. **Irja Helm.** High accuracy gravimetric Winkler method for determination of dissolved oxygen. Tartu, 2012, 139 p.
119. **Karin Kipper.** Fluoroalcohols as Components of LC-ESI-MS Eluents: Usage and Applications. Tartu, 2012, 164 p.
120. **Arno Ratas.** Energy storage and transfer in dosimetric luminescent materials. Tartu, 2012, 163 p.
121. **Reet Reinart-Okugbeni**. Assay systems for characterisation of subtype-selective binding and functional activity of ligands on dopamine receptors. Tartu, 2012, 159 p.
122. **Lauri Sikk.** Computational study of the Sonogashira cross-coupling reaction. Tartu, 2012, 81 p.
123. **Karita Raudkivi.** Neurochemical studies on inter-individual differences in affect-related behaviour of the laboratory rat. Tartu, 2012, 161 p.