OVERVIEW ON NEUROPEPTIDES

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Abstract

Neuropeptides are short sequences of amino acids which act on G protein couple receptors that work either directly or indirectly to function synaptic activity. According to the research neuropeptides are wide ranging as well as various categories of signaling molecules of our brain. Neuropeptides can also act as neurotransmitters, carry out neurotransmission. Neuropeptides are involved in various activities like pain management, depression, seizure activity, addiction, skin disease etc. Neuropeptides are one of the most diverse classes of signaling molecules which has a great interest in disease treatment. Neuropeptides are safer than bioactive molecules like proteins and monoclonal antibodies due to their less side effects and simple chemical modifications. There has been much progress in the field of neuropeptides. Few Peptide receptors have been identified, new drugs have been developed and novel insights into the regulation of peptide synthesis have been obtained. Though, the specific physiological roles of neuropeptides are needed to be researched.

Keywords: Neuropeptides Y, Hypothalamic orexigenic neuropeptide, galanin, MCH, calcitonin gene-related peptide, substance p

Dedication

I want to dedicate this project to my parents.

Acknowledgement

I would like to proceed by thanking the Almighty who is the source of our strength and knowledge which has enabled me to complete this project with full diligence.

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List of Acronyms

NPY	Neuropeptide Y
CGRP	Calcitonin gene-related peptide
VIP	Vasoactive intestinal peptide
SP	Substance P
CRTH2	Chemoattractant receptor expressed on T helper type 2
EoE	Eosinophilic esophagitis
RAMP1	Receptor activity-modifying protein 1
NGF	Nerve Growth Factor
CRH	Corticotrophin releasing hormone
GAL	Galanin
CCK	Cholecystokonin

Chapter 1 Introduction

1.1 What are neuropeptides?

Neuropeptides are short sequences of amino acids which act on G protein couple receptors that work either directly or indirectly to function synaptic activity (Li & Kim, 2009). According to the research neuropeptides are wide ranging as well as various categories of signaling molecules of our brain. Neuropeptides can also act as neurotransmitters, carry out neurotransmission, acting as autocrine and paracrine regulators in a closed cellular environment and as hormones in a long range. Around 70 genes are recognized of these neuropeptides and these are regarded as “classical neuropeptides”. These genes are divided into 18 subfamilies corresponding to their precursor or peptide structure or their functions. The study of neuropeptides has been working more than 30 years. Neuropeptides were experienced before inventing the idea of chemical signals in physiology. In the nineteenth century, extraction of organs was done and their effects on the system were explored. Secretin, oxytocin, vasopressin, and insulin were termed as hormones and encountered as biological activities in the organ system (Burbach, 2011). Mammalian neuropeptides were identified by the extraction of human intestine or brain or hypothalamic fragments in a large scale. Later the Nobel prize was awarded to Guillemin and Schally. On the other hand, new peptides are discovered from different sources like frog skin. Mutt and Tatemoto extracted more peptides from gut skin by the use of C-terminal amidation as a chemical marker (Hokfelt *et al.*, 2003). The second idea of neuropeptides was the neurosecretion which was researched by Carl Speidel in 1917 and Ernst Scharrer. Earlier it was recognized substances were present in the nerve cells that could be secreted not into the bloodstream, but internally in the nervous system. Oxytocin, vasopressin and their neurophysins were further denoted as immunocytochemistry. The third angel of neuropeptides was to determine the physiological

action of neuropeptides in the nervous system and cognitive action in the brain. David de Wied developed the idea of hormones on rat behavior and found out ACTH, MSH and vasopressin that acted on the brain and learning and memory processing, but at that time the idea of receptors was still unfound (Burbach, 2011).

1.2 Functions of Neuropeptides

Neuropeptides are involved in various activities like pain management, depression, seizure activity, addiction, skin disease etc. According to Lembeck's theory, absence of substance P and neurokinin I receptors does not show any pain on the mice. Antagonists of opioid receptors have been used to treat drug addiction. Neuropeptides may be used for the treatment of substance abuse. From the research we found that galanin is a potential neuromodulator for drug abuse management. Acupuncture is a traditional method which is used for pain management. Low frequency acupuncture releases Beta-endorphins and Met-enkephalin while high frequency releases dynorphin which causes analgesic effect to relieve pain. The research shows that neurokinin I antagonist plays an important role in depression as a serotonin selective reuptake inhibitor. Though it has some adverse impact and it is established in placebo-controlled blinded studies by Merck and Pfizer. Seizure activity can be managed by using neuropeptides. Neuropeptide Y has an inhibitory effect that is suspected of being an endogenous antiepileptic agent that can help to manage seizure activity. Neuropeptides are used for inhibiting the aging process for a very long period. Specific neuropeptides have their different receptors on epidermal cells. These neuropeptides play an important role in different skin diseases like psoriasis, atopic areata, vitiligo, hypertrophic scars etc. NPY is one of the most important peptides located in the brain which helps to regulate carbohydrate intake. It stimulates NPY-ergic arcuate paraventricular nucleus

pathway by exercise, fasting which can be followed by increasing parasympathetic activity and inhibiting sympathetic activity (Hokfelt *et al.*, 2003).

1.3 Importance of Neuropeptides

Neuropeptides are one of the most diverse classes of signaling molecules which has a great interest in disease treatment. Neuropeptides are safer than bioactive molecules like proteins and monoclonal antibodies due to their less side effects and simple chemical modifications (Panawala, 2017).

Why neuropeptides are better than classical neurotransmitter?

Neuropeptides produce in the cell body of a neuron whereas neurotransmitters produce at the axon terminal of presynaptic neurons. Neuropeptides diffusion takes time to the action site, but it shows prolonged action. On the other hand, neurotransmitters produce acute response to the action site because they are destroyed at the presynaptic cleft. Because of their different mechanism of actions after releasing, neuropeptides showed better response than the classical neurotransmitters.

1.4 Aim of the study

The aim of the study is to find out the importance of neuropeptides, new drug discovery and the importance of neuropeptides in disease treatment. Neuropeptides has become a promising sector for treating chronic disorders without showing adverse effects.

1.5 Objectives of the study

The objective of this study is to describe how neuropeptides are used in obesity treatment, anxiety and depression, skin disease and addiction. The other objective is to show the future perspectives of neuropeptides.

Chapter 2 Methodology

Thorough literature review was done to obtain all the information used in this review paper. The information was collected from various credible sources, including different peer-reviewed journals, online scholarly databases, books and newspapers. Following are the list of some of the many databases that were searched extensively for the present study.

-journal database

-Library catalogue

-Subject specific professional websites

-Newspaper database

Chapter 3 Results and discussion

3.1 Neuropeptides in obesity treatment

There are some anti-obesity drugs which are available in the market for treating obesity but have long term adverse effects on the body. The only approved long term drugs for obesity in the UK market is orlistat (Xenical) which inhibits intestinal lipases. They act on the CNS as neuromodulators of the classical neurotransmitter system. They have more receptor recognition sites which leads to greater binding affinity and higher receptor selectivity to minimize adverse effects.

Hypothalamic Orexigenic neuropeptides like NPY, Melanin-concentrating hormone, orexin, Relaxin-3, galanin like peptides antagonists are used to treat obesity. Y1 receptor antagonists have administered in animals but not in human clinical trial studies. BMS-193885 is a selective Y1 receptor antagonist which helps to reduce 8.5% body weight in 44days in animal trial. Though it has poor bioavailability and poor absorption in the intestine. It did not show any behavioral side effects so considering it for chronic use. Many Y5 receptor antagonists were designed and they showed efficacy by reducing body weight. Only two (MK-0557 and S-2367) have used in clinical trial. Further it has been stopped for using due to its side effects and didn't reduce weight combination with a hypocaloric diet for 52 weeks or with co-administration of sibutramine or orlistat for a period of 24 weeks.

Melanin-concentrating hormone receptor antagonists have received a great achievement in treating obesity but due to its side effects the system has been stopped. MCH containing fibers is widely distributed in the brain and it has various functions. It does not show any pre-seizure effects and due to the reason it can be a concern for chronic use.

Besides Hypothalamic anorectic neuropeptides like Melanocortins are now in research to find out the proper mechanism in treating obesity. 493 (formerly BIM 22493) is a small peptide agonist with high selectivity for MC4 receptors. In preclinical studies, when treated with RM-493 for 8 weeks displayed reduced 13% body weight, food intake, glucose tolerance. This study revealed the improvement of heart rate and blood pressure attributed to the increased body weight (Kievit et al, 2010). RM-493 is now in the research phase I clinical trial for obesity treatment (Rhythm Pharmaceuticals, 2011).

BDNF plays an important role in neuronal plasticity and neurogenesis. Phase iii clinical trials have shown some efficacy in treating motor neuron diseases. Therefore, no significant advantages have been reported of receiving BDNF (The BDNF Study Group, 1999). Furthermore, research is needed in this case (Boughton & Murphy, 2013).

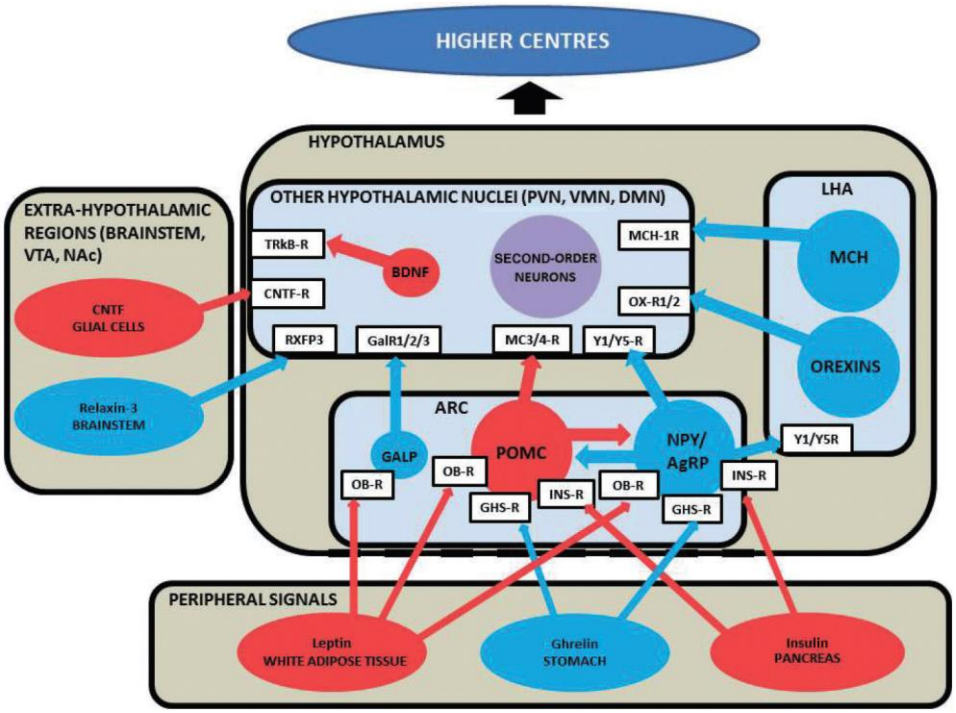

Figure 1: *Schematic diagram illustrating the neuropeptidergic signalling pathways regulating energyhomeostasis. Hypothalamic nuclei integrate and process signals of energy homeostasis from the periphery and other CNS regions and induce appropriate changes in appetite and energy expenditure. Peptides coloured blue are orexigenic, promoting food intake, while peptides coloured red are anorexigenic, promoting satiety. Receptors are in white boxes. Purple indicates neurons, which can be either orexigenic or anorexigenic. Non-peptidergic neurotransmitters are not included in this diagram for clarity. The peripheral factors in the diagram are reviewed elsewhere (Murphy et al., 2006). CNTF-R, ciliary neurotrophic factor receptor; GalR1/2/3, galanin receptors 1,2 and 3; GHS-R, growth hormone secretagogue receptor; INS-R, insulin receptor; MC3/4-R, melanocortin 3/4 receptors; NAc, nucleus accumbens; OB-R, leptin receptor; RXFP3, relaxin/insulin-like family peptide receptor 3; TRkB-R, tyrosine-related kinase B receptor; VTA, ventral tegmental area; Y1/Y5-R, neuropeptide Y1/Y5 receptors (Boughton & Murphy, 2013).*

3.2 Neuropeptides in allergic disease

The role of neuropeptides in the allergic disease has become an attractive part for the researcher in the recent time. Earlier, some neuropeptides like vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP), substance P, Neuropeptide Y, and Nerve growth factor have been implicated in the pathogenesis of the allergic diseases. Eosinophils

and mast cells accumulate in the esophagus interacting CRTH2 receptor [chemoattractant receptor homologous molecule expressed on Th2 lymphocytes with nerve-derived VIP. By inhibiting the VIP-CRTH2 axis helps to feel better for chronic EoE patients [the allergic esophageal disease termed as eosinophilic esophagitis]. This helps to treat allergic rhinitis, asthma, atopic dermatitis.

CGRP is another important neuropeptide of enteric sensory neurons that gives the microtubule reorganization in mucosal mast cells, promoting the development of food allergy. Moreover, mice deficient in Receptor activity-modifying protein 1 (RAMP1), a component of CGRP receptor, show reduced airway resistance compared to other mice. CCL17 induced CCR4-dependent CGRP synthesis in airway epithelial cells of asthmatic patients have been observed and found this can be helpful for the treatment of asthmatic patients. The anti-inflammatory potential of CGRP has been found that this neuropeptide has the ability to reduce the proliferation of antigen-specific T cells and enhance the T regulatory cells via modulation of DC. A study by Kay et al. stated that the enhanced expression of CGRP in the lesional skin as compared to the non-lesional skin in chronic spontaneous urticaria patients (Verma, 2017).

3.3 Neuropeptides in skin disease

Neuropeptides has become another attraction in the skin diseases treatment like psoriasis. It's a chronic skin condition which causes skin cells to multiply ten times faster than normal. From the previous studies we found that the role of the nervous system in chronic inflammatory disease. Nervous system works by secreting neuropeptides and neuropeptides on inflammatory cells and keratinocytes. By secreting NGF and different cytokines, the epidermis gives effect on nervous system and inflammatory cells. Neuropeptides such as SP (substance P) and CGRP (Calcitonin gene-related peptide) levels rise in the skin of psoriasis

patients which indicates that the nervous system is playing an important role to upgrade inflammation and itching. Moreover, VIP and CGRP can cause excessive keratinocyte proliferation in psoriasis and CRH may play a role in disease exacerbation caused by long-term mental stress. When the HPA axis (Hypothalamic-pituitary-adrenal axis) is activated, it can cause more glucocorticoids hindering the inflammatory response. When the HPA axis is inoperative, this anti-inflammatory effect will be weakened. HPA deficiency patients have weakened anti-inflammatory leading to amplified pro-inflammatory effects of CRH, SP, and CGRP.

Cutaneous denervation is a promising sector which helps to improve psoriasis which plays an important role in cancelling psoriasis. Though, the mechanism is not fully clear. Either VIP is more pro- or anti-inflammatory, it is still in research (Zhang *et al.*, 2020).

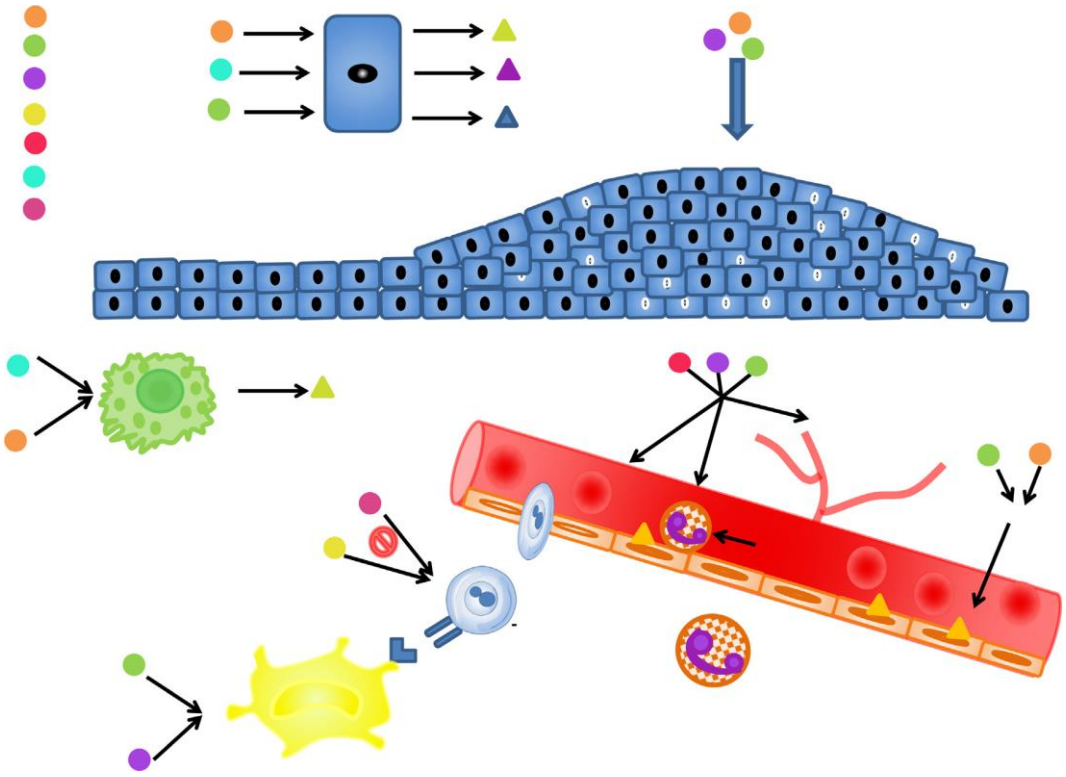

Figure 2: Mechanism of neuropeptides in psoriasis (Zhang et al., 2020).

Table 1: Mechanism of neuropeptides in psoriasis (Zhang et al., 2020).

Neuropeptide	Mechanism
Substance P	Promote mast cell degranulation, elevate the level of E-selectin, and stimulate the proliferation and secretion of IL-1,TNF-alpha, NGF of keratinocyte
Calcitonin-related gene(CGRP)	Increase E-selectin levels, promote neutrophil, T cells, dendritic cells in filtration, bias Langerhans cells to present antigens to Th17, promote the proliferation of keratinocyte, increase the expression of VEGF mRNA and promote angiogenesis, lead to IL-17 secretion of Th17.
Corticotrophin releasing hormone(CRH)	Increase the secretion of IL-6 in keratinocytes, enhance the role of Th1 cells, stimulate mast cell degranulation, and reduce the expression of IL-1β, IL-18, and VEGF in keratinocytes
Alpha-melanocyte stimulating hormone (α-MSH)	Induce DCs to produce Tregs, inhibit Th17 cell proliferation and secretory function, increase the level of IL-10, decrease the level of IL17, IL-22, and inhibit the production of adhesion molecules
Vasoactive intestinal peptide(VIP) and	PACAP: Cause vasodilation and plasm

Pituitary adenylate cyclase activating peptide(PACAP)	extravasation, chemotactic leukocyte, promote angiogenesis, induce Langerhans cells to present antigens to Th17, and enhance plaques in psoriasis. VIP: promote proliferation of keratinocyte and increase the level of IL-6 and IL-8, promote angiogenesis
Cholecystokonin(CCK)	Inhibit the production of IL-6, downregulate the level of regulatory cells, inhibit the differentiation of Th17 and the production of IL17, IL-22, and reduce itching
Galanin(GAL)	Promote angiogenesis, chemotactic leukocyte

3.4 Neuropeptides in depression and anxiety

Some neuropeptides play a significant role in treating depression. Previously, the monoamine hypothesis was used for treating depression all over the world. Antidepressants using monoamine hypotheses are not giving proper therapeutic effects up to 30% of the patients who are suffering. These antidepressant drugs take time 3–4 weeks before the onset of clinical efficacy. Besides, the cellular and molecular events which cause depression are still unknown. Some important neuropeptides like substance P, corticotropin-releasing factor, neuropeptide Y, vasopressin, and galanin have some evidence to treat anxiety and depression. Evidence shows that neuropeptides have discrete localization compared to standard antidepressants. These neuropeptides show adverse effects compared to those neuropeptides. It is now clear that neuropeptides closely interact with monoamines though the mechanism is still unclear.

They are still working to find out small molecule ligands with higher affinity and selectivity for understanding the functional interaction between neuropeptides and monoamines (Madaan, 2009).

Neuropeptides injection reduces the symptoms of the disease or psychopathology of a disease. Like, CCK4 infused patients are reported to reduce panic attack whereas OT infused patients are found to reduce repetitive behaviors in adults who have autistic and Asperger's disorders. Pharmacological treatments of mental diseases reduce modifications in the activity or the concentration of neuropeptides. Example, the anxiolytic benzodiazepines was found to reduce the activity of CCK in the brain (Surget et al, 2006).

3.5 Neuropeptides in addiction

Neuropeptides are used for treating addictive behaviors and may provide new therapeutic target for the treatment of drug addiction and other substances. Drug and alcohol induced alterations of neuropeptides systems within the extended amygdala contributes effects to the negative affective state during the withdrawal of alcohol or other substances. Negative affective state promotes taking drugs as well as to reduce pain reflecting excessive drug and alcohol intake stemming from dependence (Koob & Le Moal, 2001). Neuropeptides have been proposed to influence their intake, drug's intoxicating effects (Schuckit, 2009) and the modulation of pain (Thiele, 2017).

The increasing number of genetic studies showed linkage between diseases and neuropeptide signaling which can be emphasized to many neuropeptide receptor ligands as drugs. In clinical indications it was thought that pharmacology of the neurotransmitters cannot be described properly like control of excitability, major depression whereas neuropeptide receptors become obvious drug targets. Many new neuropeptides are using to find new medications although failures will occur. e.g., NPY5 in obesity or NK1 in

pain/anxiety/depression did not fulfill their target. Again, it is showed that less than ten years after the discovery of the orexins and their receptors in 1998, clinical phase II data were published in 2007 for an orexin dual antagonist almorexant and suvorexant is completing phase III studies in primary insomnia in 2012 (Hoyer & Bartfai, 2013).

Chapter 4 Future studies

After the discovery of substance P and intense research on neuropeptides, the first peptide drug, substance P antagonists has been clinically tested for the treatment of mood disorder. The antagonists are effective as selective serotonin reuptake inhibitors and have less side effects. This drug has been approved by FDA for emesis treatment after chemotherapy. There are some difficulties in research and that is synthesis of selective and potent BBB-penetrant agonists or antagonists (Hökfelt et al, 2003). When these difficulties will be overcome this drug can be used to treat cognition, seizures, reward behavior, mood and appetite. There are various receptors of neuropeptides in the CNS and PNS. Many drugs have been discovered after the discovery of neuropeptides but these drugs do not act selectively on these receptors from the clinical research. To overcome these difficulties HTS technology is needed to accelerate the discovery of new drugs like non-peptide antagonists and agonists (Poyner et al., 2000).

Chapter 5 Conclusion

There has been much progress in the field of neuropeptides. Few Peptide receptors have been identified, new drugs have been developed and novel insights into the regulation of peptide synthesis have been obtained. Though, the specific physiological roles of neuropeptides are needed to be researched. These features and the neuropeptides and neuropeptide receptors provide many opportunities to discover new drugs in treating various diseases like depression, obesity, skin diseases etc**.** Not only have that various number of questions arisen to carry out targeted drug development in an effective way. Using different advanced tools and using animal models, it should be possible to answer all these questions in an effective way.

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