



## THE PATHWAYS INVOLVED IN TLQP-62 MEDIATED BIOLOGICAL FUNCTIONS: A REVIEW

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### ABSTRACT

VGF is neurosecretory protein, belongs to the extended granin family of proteins, originally recognized as a nerve growth factor (NGF) inducible gene product. It is selectively synthesized mostly in neuronal and neuroendocrine cells. It has a very simple organization. It has several biologically active peptides, described as VGF-derived peptides including APPG-40, APPG-37, GRPE-37, NERP-1, NERP-2, NAPP-129 (VGF 20), VGF 18, HFHH-51 (VGF 6), HHPD-1, AQEE-30 (Peptide V), LQEQ-19, TLQP-21 and TLQP-62 (VGF 10). Among these peptides TLQP-62 is of great significance because of its several remarkable biological effects like regulation of memory formation, glucose homeostasis and insulin secretion, gene transcription, neurogenesis, etc. Although in light of these outstanding behavioral and physiological roles, very few information are available regarding its mode of action. Still, no receptor has been found for TLQP-62 mediated cascading pathways. However, considering the significant biological function of TLQP-62, this review study was done to summarize the pathways involved in TLQP-62 mediated action, based on the information till now. TLQP-62 activates signal transduction cascades by two mechanisms including opening of the calcium channels and modulating the AMPK, PKC and ERK pathways. These events result in an increase in intracellular  $Ca^{2+}$  concentration which permits the secretion of insulin. TLQP-62 would be a significant therapeutics and drug discovery tool for a wide range of diseases to which the peptide related, if TLQP-62 mediated pathways are known, as a whole.

**Indexing terms/Keywords** : TLQP-62, VGF, Granin Family, Signaling Pathway

**Subject Classification** : Biotechnology, Receptorology

**Type (Method/Approach)** : Review

Language : English

Date of Submission : 2017-12-28

Date of Acceptance : 2018-01-02

Date of Publication : 2018-02-28

ISSN : 2348-6201

Volume : 07 Issue : 01

Journal : Journal Of Advances In Biotechnology

Publisher : CIRWORLD

Website : <https://cirworld.com>



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## INTRODUCTION

VGF is a Nerve Growth Factor (NGF) responsive gene. It has a non-acronymic name. It is not similar to VEGF (vascular endothelial growth factor). This gene was first discovered in 1985 in an experiment with PC12 cells. The organization of the VGF is relatively simple and it has a single copy gene consisting of few kilobase pairs [1,2].

VGF has diverse physiological functions including the following: modulation insulin secretion and glucose homeostasis, regulation of energy homeostasis, regulation of gastrointestinal function, regulation of hormone, neurotrophin, and/or neurotransmitter release, regulation of sexual function, regulation of body fluid homeostasis, modulation of pain, regulation of emotion/psychiatric disease, proliferation of neural progenitor cells (NPCs), memory formation in Hippocampus cells, neuroprotection, drug discovery as a potential diagnostic tools and/or targets, antidiabetic drug discovery programs etc.

There are several VGF derived peptides including APPG-40, APPG-37, GRPE-37, NERP-1, NERP-2, NAPP-129 (VGF 20), VGF 18, TLQP-62 (VGF 10), HFHH-51 (VGF 6), HHPD-1, AQEE-30 (Peptide V), LQEQ-19 and TLQP-21. Among these VGF derived peptides, the two peptides namely TLQP-21 and TLQP-62 with TLQP are sequenced individually at N-terminus with total length 21 and 62 amino acids [3]. Between them, TLQP-62 is the subject of interest in this present study.

Out of several bioactive peptides derived from VGF, TLQP-62 is of great importance because of its various behavioral and physiological roles and functions. TLQP-62 plays a critical role in the modulation of insulin secretion and glucose homeostasis, regulation of gene expression, regulation of memory formation in Hippocampus cells, proliferation of Neural Progenitor Cells (NPCs) by activating the signaling pathways downstream of the glutamate receptor and TrkB-BDNF-dependent mechanism.

Other behavioral and physiological roles of this neuropeptide includes the following: stimulation of insulin secretion in insulinoma cells, regulation of gene expression in INS1E cells, activation of the intracellular signaling pathways, enhancement of intracellular calcium mobilization in INS1E cells, trophic and proliferative effects on insulinoma cell lines, in the act of improving glucose tolerance in vivo, determination of phases of neurogenesis, enhancing synaptic activity [4-6].

More specifically, the proliferation of NPCs can be induced by two different processes including TrkB-BDNF-dependent signaling mechanism and the downstream signaling pathways of the glutamate receptors, mGluR5 and NMDA, including PKD and CaMKII. These events can be accomplished by the modulation of TLQP-62. In addition, TLQP-62 regulates memory development in the Hippocampus cells via TrkB-BDNF-dependent mechanism.

Therefore, this review study has been conducted to establish the possible mechanisms involved in the functions of TLQP-62.

## VGF

VGF gene was primarily determined as a Nerve Growth Factor (NGF) responsive gene. It is not similar to VEGF (vascular endothelial growth factor). One should not mistake one thing for another. The treatment of PC12 cells with NGF was used to make identical copies of NGF33.1, a nervous system-specific mRNA.

At first nucleic acid and amino acid sequences of the NGF33.1 cDNA clone was interpreted. Following the interpretation, this clone resembling to the NGF-inducible mRNA as VGF was assigned by Levi and his co-workers [1].

The word 'VGF' originated from the selection of this clone from plate **V** of the nerve **G**rowth **F**actor induced PC12 cell cDNA library[1, 2].



**Table 1. Basic information of VGF.**

Number of amino acids (AA) and calculated molecular mass (MM) of the pre-protein, number of amino acids and calculated molecular mass of the mature protein, observed molecular mass of the mature protein, number of dibasic sites, number/content of proline, number/content of glutamate, calculated (calc) and observed (obs) pI, and secondary structure (percent  $\alpha$ -helix) are shown for human (h), ND, not determined [7].

	Preprotein		Mature protein			Dibasic sites <sup>h</sup>	AA/% proline <sup>h</sup>	AA/% glutamate <sup>h</sup>	pI calc <sup>h</sup> /obs	% $\alpha$ -Helix <sup>h</sup>
	AA <sup>h</sup>	Calculated MM <sup>h</sup> (kDa)	AA <sup>h</sup>	Calculated MM <sup>h</sup> (kDa)	Observed MM (kDa)					
VGF	615	67	593	65	90 <sup>h</sup>	10	77/12.5	97/15.8	4.5/ND	39

VGF is different in nature within gene products that are directed with immediate early or delayed early kinetics, such as c-fos. Neurotrophic growth factors strongly and selectively caused to arise VGF [8].

The brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3) increased the cellular response of VGF in responsive neuronal targets such as cortical or hippocampal neurons [9].

As VGF derived peptide TLQP-62 is the subject of interest in this study, the comparison between TLQP-62 (Human and Rat) VGF sequence is also illustrated distinctly in Figure 1.

**TLQP-62 (Human, Rat) Sequence Comparison**

Rat				<b>TLQ</b>	<b>PPASSRRRH</b>	<b>F</b>	<b>568</b>
Human				<b>TLQ</b>	<b>PPSALRRRH</b>	<b>Y</b>	<b>566</b>
Rat	<b>569</b>	<b>HHALPPARH</b>	<b>PDLEAQARRA</b>	<b>QEEADAEERR</b>			<b>598</b>
Human	<b>567</b>	<b>HHALPPSRHY</b>	<b>PGREAQARRA</b>	<b>QEEAEAEERR</b>			<b>596</b>
Rat	<b>599</b>	<b>LQEQEELNY</b>	<b>IEHVLLHRP</b>			<b>617</b>	
Human	<b>597</b>	<b>LQEQEELNY</b>	<b>IEHVLLRRP</b>			<b>615</b>	

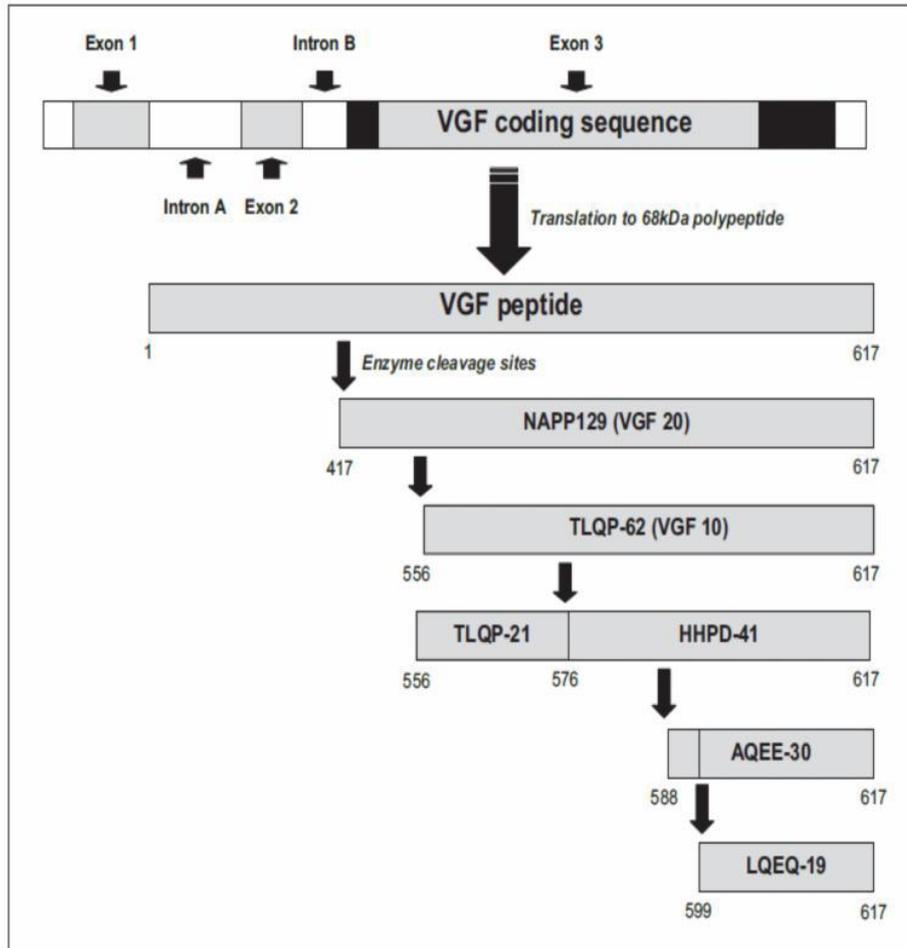
**TLQP-62 (Human) Sequence**

**TLQ PPSALRRRH Y HHALPPSRHY PGREAQARRA QEEAEAEERR LQEQEELNY IEHVLLRRP**

**TLQP-62 (Rat) Sequence**

**TLQ PPASSRRRH F HHALPPARH PDLEAQARRA QEEADAEERR LQEQEELNY IEHVLLHRP**

**Figure 1. The comparison between TLQP-62 (Human and Rat) VGF sequence. (generated from [10, 11]).**



**Figure 2. Diagrammatic view of the VGF gene and its derived peptides (mouse).** The simple structure of the VGF encodes a 617-amino acid protein in rats, and a 615-amino acid protein in humans. Both of the amino acid proteins are cleaved into various peptides. The region encoding the 5' untranslated sequence of VGF are interrupted by two introns. And exon 3 encodes the whole VGF protein. The portions of the VGF gene have potential to demonstrate biological activity, listed in Table 2 [3].

**Table 2. A chart of the VGF derived peptides.** The first four amino acids, in short TLQP (Thr-Leu-Gln-Pro) generalizes the nomenclature of the peptides by its length. For instance: The two peptides namely TLQP-21 and TLQP-62 with TLQP are sequenced individually at N-terminus with total length 21 and 62 amino acids. However, this excludes the neuroendocrine regulatory peptides, NERPs and VGF 20 which is 20 kDa gene, based on the apparent molecular weights pointed out by Western blot analysis method. (Adapted from [3]).



Peptide names	Amino acid position on VGF precursor	Length	Biological activity known?
APPG-40	23-62	40	No
APPG-37	23-59	37	No
GRPE-37	26-62	37	No
NERP-1	302-309	8	Yes
NERP-2	341-350	10	Yes
NAPP-129 (VGF 20)	417-617	201	No
VGF 18	Unknown	---	No
<u>TLQP-62 (VGF 10)</u>	556-617	62	Yes
HFHH-51 (VGF 6)	567-617	51	No
HHPD-41	576-617	41	No
AQEE-30 (Peptide V)	588-617	30	Yes
LQEQ-19	599-617	19	Yes
TLQP-21	556-576	21	Yes

### TLQP-62 as a functional VGF derived peptide

Among other bioactive VGF derived peptides, TLQP-62 is of great interest and it is significant for its various behavioral and physiological roles and functions. The role of TLQP-62, as bioactive VGF derived bioactive peptides includes the following: modulation of insulin secretion and glucose homeostasis, improving glucose tolerance in vivo, regulation of gene expression [5], regulation of memory formation [4], determining phases of neurogenesis, enhancing synaptic activity, proliferation of neural progenitor cells (NPCs) or TLQP-62 induced neurogenesis [6].

**Table 3. Biological functions of TLQP-62, at a glance.**

Functions	Reference
stimulating insulin secretion in insulinoma cells	[5, 12,13].
regulation of gene expression in INS1E cells	[5, 14, 15, 16].
activating the intracellular signaling pathways	[5, 17].
increasing intracellular calcium mobilization in INS1E cells	[5, 18].
determines phases of neurogenesis	[6, 19, 20, 21, 22, 23].
enhances synaptic activity	[6, 24, 25].
induced proliferation of Neural Progenitor Cells (NPCs) by activating the signaling pathways downstream of the glutamate receptor	[6].
Regulation of memory formation in Hippocampus cells	[4, 6, 24,26,27, 28].



## PATHWAYS OF TLQP-62 MEDIATED BIOLOGICAL FUNCTIONS:

### Mechanism of TLQP-62 modulating insulin secretion and glucose homeostasis

It was previously described that TLQP-62 was pivotal for insulin secretion in insulinoma cells. [5]. And the proper control of insulin secretion was absolutely necessary for glucose homeostasis. The cells of the islet of Langerhans as well as the intramural and autonomic neurons secreted the paracrine and autocrine molecules. The release of different hormones that modulate insulin secretion was controlled by these molecules. In pancreatic islets, a diverse cargo of peptides and several biologically active molecules was found in the secretory granules of  $\beta$  cells. It suggested that the secretion of insulin and the release of these molecules were generated because of the same stimuli [29]. As  $\beta$  cells were involved in the production of VGF-derived C-terminal peptides [16], their ability to stimulate insulin secretion was evaluated. It was analyzed that TLQP-62 fragment was the most effective among other C-terminal VGF derived peptides. In the presence of low glucose, TLQP-62 caused strong induction of insulin secretion in vitro. This dose dependent stimulatory effect also potentiated GSIS. The intracellular calcium mobilization, an event dependent on calcium influx from both the extracellular compartment and intracellular storage was rapidly enhanced by TLQP-62 [18]. As the calcium influx was decreased in glucose-free medium the mechanism of action of TLQP-62 in the presence of a physiological glucose level (4.5 mM). As reported previously, under the low-glucose condition TLQP-21 did not robustly enhance insulin secretion but produced a positive effect on GSIS in the INS1E cell line among the other analyzed VGF peptides [30]. Though other C-terminal-derived peptides including AQEE-30 and LQEQ-19 but not YIEH-10 had the capacity to stimulate insulin secretion. But, the full length C-terminal TLQP-62 peptide was more potent than these peptides. However, these peptides ligate to a yet to be identified receptor(s). In previous study it has been described that TLQP-21 ligates to the C3a receptor [31,32]. The C3a and TLQP-21 with an intact C-terminal Ala-Arg moiety activated the C3aR1 that was not present in TLQP-62. Furthermore, the pro-secretory effect of TLQP-62 was blocked or inhibited by C3aR1 antagonist SB290157. So, it was contemplated that a different receptor was activated by TLQP-62. As both the AQEE-30 and LQEQ-19, possess some biological activity, they are not excluded. Because they may also be active on that receptor. It gives the impression that further structural and pharmacological studies are required to identify the receptor for C-terminal TLQP-62 peptide [5].

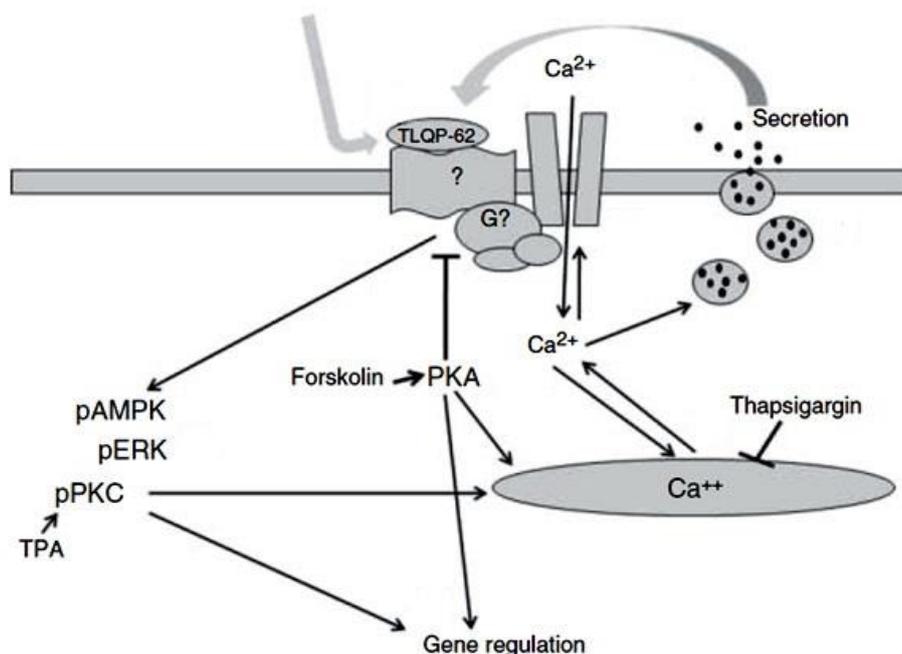


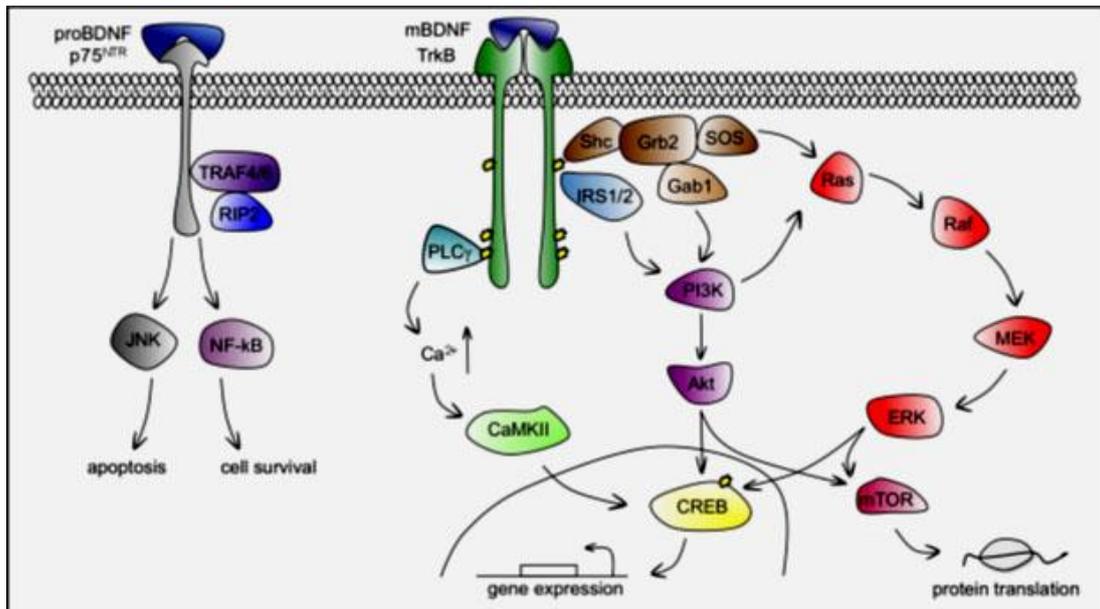
Figure 3. Mechanism of TLQP-62 modulating insulin secretion and glucose homeostasis [5].



During its mode of action, the released TLQP-62 ligates an unidentified receptor. Then the signal transduction cascades was activated by first opening calcium channels on the membrane and stimulating the endoplasmic reticulum release (ERK). Then TLQP-62 quickly activated the intracellular signaling pathways within 2–5 min and produced reversible dephosphorylation of AMPK followed by phosphorylation of ERK and PKC. The involvement of AMPK in insulin secretion caused a fast down-regulation of p-AMPK which mimics the effect of high intracellular ATP by inducing the cell to secrete insulin and by closing the potassium channels. PKA-substrate phosphorylation was not activated by TLQP-62. The fast effect of TLQP-62 on calcium was weakened by Forskolin (Frsk) which also activates the PKA pathway. On the contrary, TLQP-62-induced calcium mobilization was not inhibited by TPA which activates and then down-regulates PKC. Both these pathways, Forskolin (Frsk) activated PKA pathway and TPA activated PKC pathway were involved in increasing the intracellular  $Ca^{2+}$  concentration which was permissive for insulin secretion. However, the presence of thapsigargin inhibits the secretion of insulin. In addition, TLQP-62 is released along with insulin and a paracrine effect was exerted. Both these pathways were also involved in gene regulation which implies that TLQP-62 also has an effect on gene transcription [5].

### BDNF-dependent signaling pathways and their targets related to the neuropeptide TLQP-62

In the BDNF-dependent signaling pathways, the BDNF was bound with TrkB having high affinity. This high affinity is necessary to induce its dimerization and autophosphorylation of tyrosine residues in the cytoplasmic kinase domain. This domain served as the docking sites for effector molecules. The activation of three main signaling pathways: PLC $\gamma$ , PI3K and ERK cascades was also elicited by these docking sites eventually leading to the phosphorylation and activation of the transcription factor CREB.

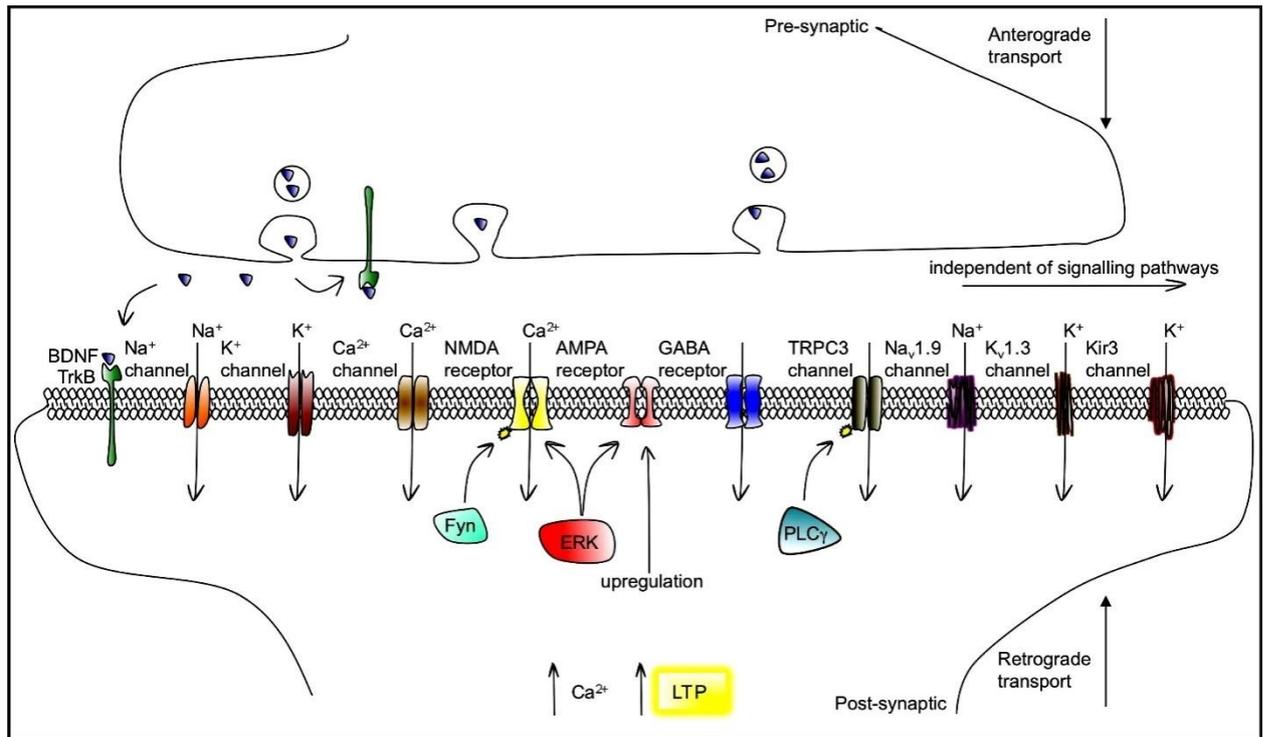


**Figure 4. BDNF–TrkB and BDNF–p75NTR signalling pathways [33].**

The transcription of genes essential for the survival and differentiation of neurons was mediated by the transcription factor CREB. The intracellular  $Ca^{2+}$  levels were increased due to the recruitment of PLC $\gamma$ . Thus, leading to the activation of CaMKII to phosphorylate CREB. PI3K was also activated via the Shc/Grb2/SOS complex through Gab1 and by IRS1/2. The activated PI3K, the phosphatidylinositides, generated lipid products that were bound with protein kinase Akt. These products also activated protein kinase Akt, upstream of CREB. Both by the Shc/Grb2/SOS complex and by PI3K, the signaling pathways of ERK can be activated. CREB phosphorylation was directly



pointed out by the phosphorylation of ERK. The mTOR was activated both by Akt and ERK which was responsible for enhanced translation initiation [33].



**Figure 5. Actions of BDNF/TrkB on different ion channels mediating fast and slow synaptic transmission in neurons [33].**

TrkB receptors can be activated by both pre- and postsynaptically by BDNF; when it (BDNF) is transported anterogradely and retrogradely. Different ion channels including  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{K}^+$  channels, within a range of seconds to minutes was modulated or activated due to the association of BDNF with TrkB through the intracellular signaling pathways. The glutamatergic neurotransmission was enhanced by BDNF by increasing open probability of NMDA (by the promotion of its phosphorylation, via Fyn-dependent and Fyn-independent mechanisms) and by the upregulation of AMPA expression. Both in NMDA and AMPA gating, the signaling cascade of ERK was involved [33].

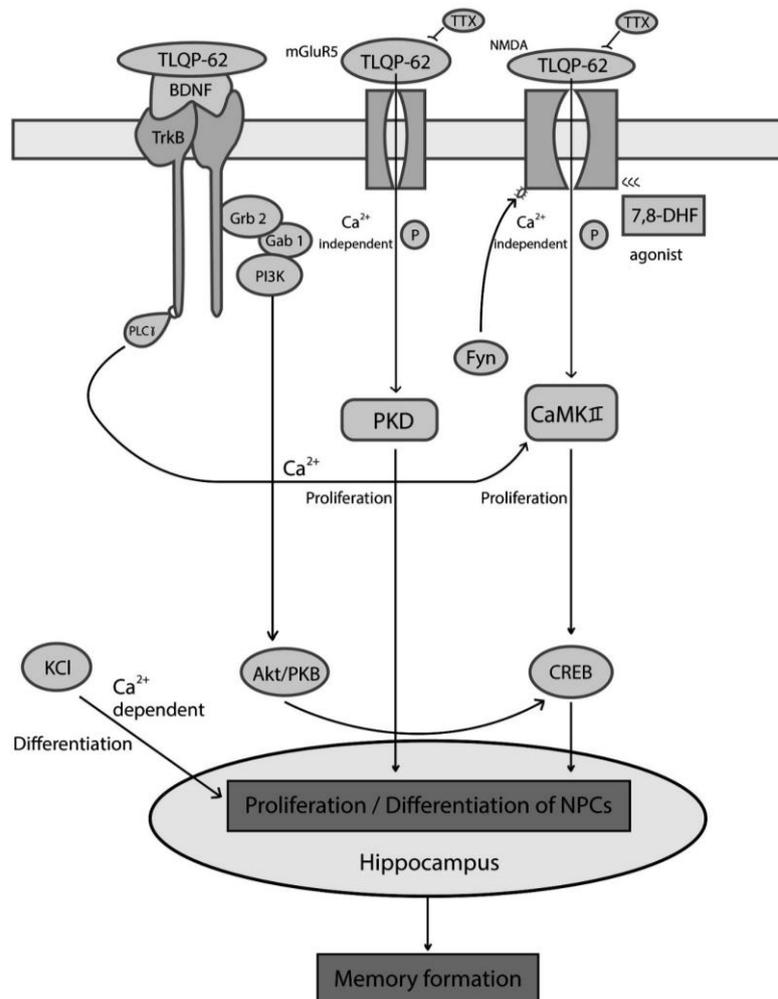
#### **Pathways of TLQP-62-induced proliferation of neural progenitor cells**

Previously the downstream signaling events activated by TLQP-62 to mediate its actions on neurogenesis was examined. It was found that the neuropeptide TLQP-62 enhances the generation of early progenitor cells in the dentate gyrus. TLQP-62 activated the Signaling pathways downstream of the glutamate receptors, mGluR5 and NMDA, include PKD and CaMKII. These receptors are mandatory for TLQP-62-induced proliferation of neural progenitor cells (NPCs). Additionally, PKD phosphorylation was identified as a key downstream event after TLQP-62-mediated activation of mGluR5 receptors in NPCs. It was also suggested by earlier data that CaMKII was functional as a potential downstream target of NMDA receptors. It was also activated in NPCs after TLQP-62 treatment. From these findings it was evident that both the mGluR5 and NMDA receptors are required for TLQP-62-induced proliferation of NPCs[6].

The relationship between TLQP-62 and BDNF signaling was also studied recently. This experiment suggested that both the TrkB phosphorylation and Trk activation is required for TLQP-62-induced proliferation of NPCs, which was also promoted by TLQP-62. However, TLQP-62-induced proliferation of NPCs was inhibited by TTX which is supported by the fact that NPCs possess  $\text{K}^+$  and



Na<sup>+</sup> channels [34, 35]. Conversely, neurogenesis was promoted by KCl in a Ca<sup>2+</sup>-dependent manner [25]. On the other hand, TLQP-62 is independent of Ca<sup>2+</sup> channel activation. Although the findings are dissimilar it may be adjusted as proliferation of NPCs was promoted by TLQP-62 while differentiation phase of neurogenesis was enhanced by KCl[6].



**Figure 6. Mechanism of TLQP-62-induced proliferation of neural progenitor cells. (generated from [6]).**

### **TLQP-62 regulating memory formation in hippocampus via a BDNF-TrkB-dependent mechanism**

After TLQP bound BDNF ligand induced activation of specific receptors TrkB, the two subunit of the receptor get dimerized. Upon dimerization, PI3K can be activated by two mechanisms. First, a phosphorylated Y residue on the receptor TrkB serves as a docking site for the p85 regulatory subunit of PI3K. This recruits the catalytic subunit of PI3K, p110, to this complex. Alternatively, upon activation of the TrkB receptor by TLQP-62 ligand, the Shc protein binds the receptor to enable the Grb-2 and Sos proteins to form a complex which results in the activation of Ras. Ras is then able to induce the membrane translocation and activation of the p110 subunit of PI3K. Activated PI3K converts phosphatidylinositol 4, 5 biphosphate (PIP<sub>2</sub>) into phosphatidylinositol 3, 4, 5 phosphate (PIP<sub>3</sub>) which results in the membrane localization of phosphoinositid dependent kinase-1 (PDK1) via its pleckstrin homology (PH) domain. Akt is also recruited to the lipid-rich plasma membrane by its PH domain and is phosphorylated at residues by T308 and S473 by PDK1 and an unidentified kinase, respectively. Akt is the primary mediator of PI3K-initiated signaling; it has a number of downstream



substrates. It is worth noting that Akt can cause the activation of specific substrates (e.g. CREB) which results in the transcription of genes responsible for memory formation [4].

### AN INTEGRATED MODEL OF THE ABOVE STUDY

An integrated model based on the above study can be drawn which illustrates the cascading pathways of TLQP-62 correlating with its functions:

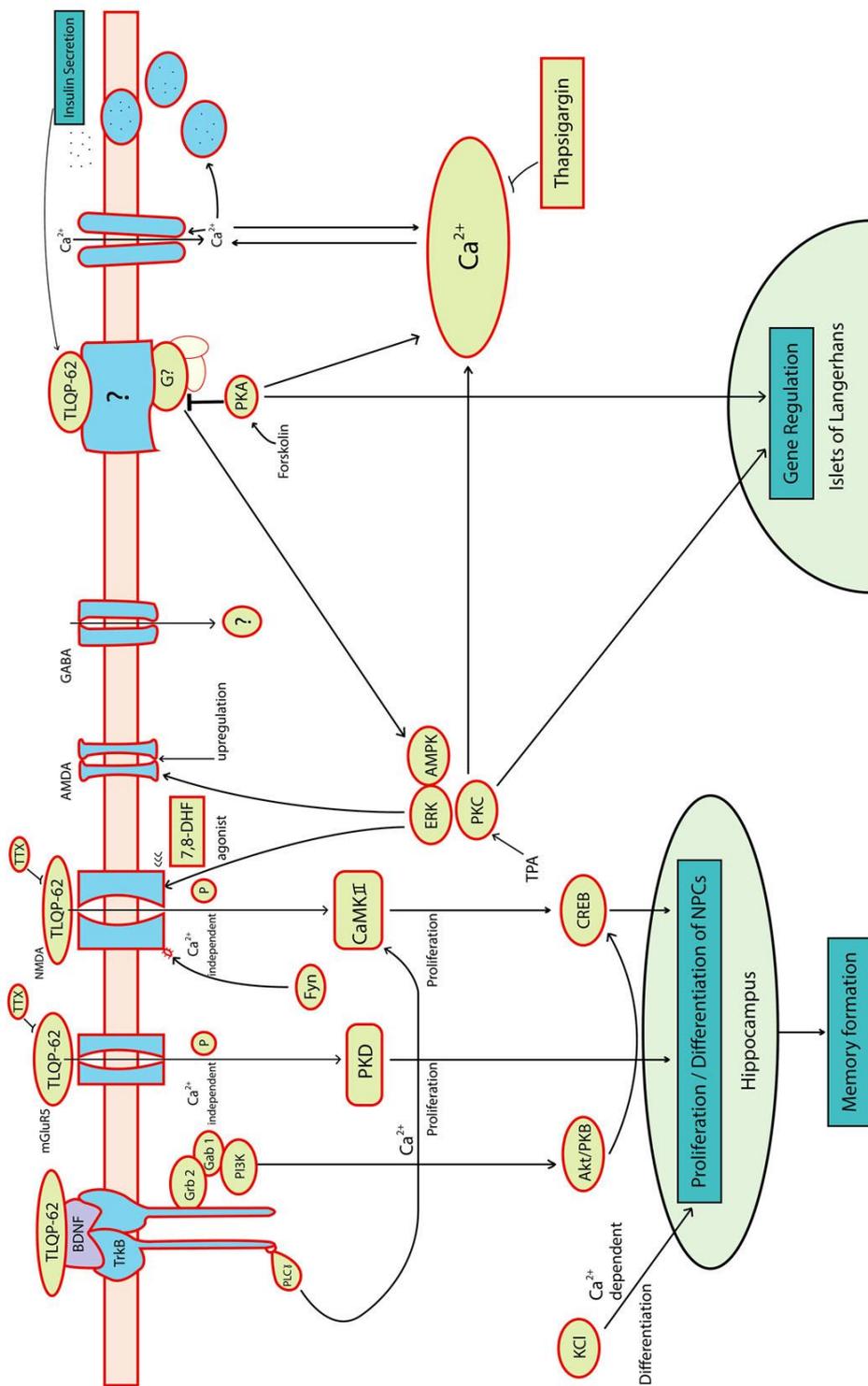


Figure 7. An integrated proposed model illustrating the cascading pathways of TLQP-62 correlating with its functions.



## DISCUSSION

This review study demonstrates that impaired insulin secretion and insulin resistance play major roles in the development of Type 2 Diabetes. In the  $\beta$  cells and other cell types of the islet of Langerhans, VGF derived peptide was present [16,30, 36]. One of the VGF peptide, namely TLQP-62 plays a critical role in the modulation of insulin secretion. Primarily, the released TLQP-62 ligates to a still unidentified receptor. Then, signal transduction pathways activate by two mechanisms. At first, calcium channels are opened on the membrane. Following the stimulation of endoplasmic reticulum release (ERK), then secondarily by modulating the AMPK, PKC and ERK signaling cascades, the increase of intracellular  $Ca^{2+}$  concentration has been observed. This permits the secretion of insulin. Along with insulin, TLQP-62 is also released. TLQP-62 also has an effect on gene regulation [5].

The neuropeptide, TLQP-62 also enhances the generation of early progenitor cells in the dentate gyrus. TLQP-62 induced proliferation of NPCs requires glutamate receptors, mGluR5 and NMDA. TLQP-62 activates the signaling pathways downstream of the glutamate receptors including PKD and CaMKII. These receptors are essential for TLQP-62 induced proliferation of NPCs. While TLQP-62 promotes the proliferation of NPCs, KCl enhances the differentiation phases of neurogenesis. In addition, the signaling pathways downstream of these glutamate receptors activated by TLQP-62 are  $Ca^{2+}$  independent. On the contrary, KCl promotes neurogenesis in a  $Ca^{2+}$  dependent way. This study also describes the relationship between TLQP-62 and BDNF signaling. TLQP-62 activates BDNF-receptor TrkB in vitro. More specifically, TLQP-62 promotes the phosphorylation of TrkB and the activation of Trk. These events are absolutely necessary for TLQP-62 induced proliferation of NPCs [6].

BDNF-TrkB dependent signaling plays a significant role in cognition function including memory formation [28, 37,38]. TLQP-62, acts as a modulator of fear memory consolidation in the adult hippocampus. Additionally, TLQP-62 induces acute and transient TrkB receptor activation, which in turn regulates memory consolidation [4].

This review study investigates the possible mechanisms of TLQP-62 and also some of the existing and possible functions of this neuropeptide. Although some of the functions and signaling cascades of the TLQP-62 have already been established, the receptor of the TLQP-62 is yet to be identified.

The eventual identification of the TLQP-62 receptor will create enormous opportunities for future research and experiment. The discovery of TLQP-62 receptor and other interacting molecules and proteins will bring some other elements to light that are involved in the mechanisms and functions of TLQP-62.

Further study on TLQP-62 mediated neurogenesis will give additional insight into the process like synaptic activity, learning and memory development as well as disease like psychiatric disorders. TLQP-62, as a new insulin secretagogue, improves glucose tolerance. Thus, it is essential for future antidiabetic drug discovery programs.

Considering the several biological activities of the TLQP-62 like regulation of memory formation, glucose homeostasis and insulin secretion, gene transcription, neurogenesis, etc., it can be concluded that the peptide TLQP-62 could be a significant therapeutics and drug discovery tool for a wide range of diseases like diabetes, psychiatric disorders, if TLQP-62 mediated pathways are known, as a whole.

## RECENT UPDATE

It was a big challenge to detect the receptors of VGF-derived peptides which clear up their cellular signalling mechanisms, but yet no receptor for TLQP-62 has been unveiled. Till now three receptors of TLQP-21 have been identified by using powerful proteomic methods: HSPA8 [39, 40], C3AR1 [32],



and gC1qR [41, 42]. Among these receptors, C3AR1 and gC1qR are receptor were of rodent model, on the contrary, upto now HSPA8 is a receptor (first putative) for human model [32, 39, 40, 42].

### **FUTURE PERSPECTIVE**

Future studies are of much interest to reveal the entire illustration of physiologically essential receptors/binding proteins/partners with further characterization of receptor dependent and/or receptor-independent signaling pathways what is of topmost importance in the field of VGF. Additionally, this neuropeptide can be contemplated as a recent, powerful, robust insulinotropic peptide. In future, TLQP-62 can be used as a potential antidiabetic drug for medicinal purposes amongst other purposes.

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