

MANAGEMENT OF INSULIN-DEPENDENT DIABETES MELLITUS BY PREPROINSULIN AND PREPROTACHYKININ SUBSTANCE P: EXPERIMENTAL EVIDENCE AND THERAPEUTIC PROSPECTS

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ABSTRACT

Insulin-dependent diabetes mellitus (IDDM), currently termed type 1 diabetes mellitus (T1DM), is a chronic autoimmune disorder characterized by immune-mediated destruction of pancreatic β -cells, progressive loss of endogenous insulin secretion, and lifelong dependence on exogenous insulin. Although modern insulin formulations and glucose-monitoring technologies have substantially improved disease management, they do not directly eliminate β -cell autoimmunity or regenerate functional β -cell mass. Consequently, therapeutic strategies aimed at restoring endogenous insulin production while simultaneously modifying the pancreatic inflammatory microenvironment have attracted considerable interest. Two experimental approaches relevant to this objective are preproinsulin-based gene therapy and modulation of the preprotachykinin–Substance P pathway. Preproinsulin gene delivery attempts to provide non-pancreatic tissues with the genetic information required for sustained insulin production, whereas Substance P (SP), an 11-amino-acid tachykinin derived principally from the TAC1/preprotachykinin-A precursor, influences neuroimmune signaling, pancreatic inflammation, insulin sensitivity, and β -cell survival. Experimental studies in non-obese diabetic (NOD) and streptozotocin-induced diabetic mice demonstrate that Substance P can reduce insulinitis, preserve pancreatic β -cell mass, promote compensatory β -cell proliferation, and delay hyperglycemia. Separately, preproinsulin gene-transfer studies have demonstrated prolonged production of insulin-like peptides and improvement of hyperglycemia in diabetic rodents. This review discusses the pathophysiological basis of T1DM, preproinsulin biosynthesis, preprotachykinin/Substance P biology, experimental evidence for their therapeutic effects, possible complementary mechanisms, limitations, and translational prospects. Importantly, preproinsulin and Substance P should presently be regarded as two complementary experimental strategies rather than an established combined treatment for T1DM.

KEYWORDS: Type 1 diabetes mellitus; IDDM; preproinsulin; preprotachykinin; TAC1; Substance P; NK1 receptor; pancreatic β -cell; NOD mouse; gene therapy.

1. INTRODUCTION

Type 1 diabetes mellitus is an autoimmune metabolic disorder in which autoreactive immune cells progressively destroy insulin-producing β -cells of the pancreatic islets. Loss of β -cell mass ultimately produces severe insulin deficiency, hyperglycemia, and dependence on exogenous insulin.

Insulin replacement remains essential for patients with established T1DM. Nevertheless, conventional insulin therapy substitutes for the final hormonal deficiency rather than correcting the immune and cellular mechanisms responsible for β -cell loss. Consequently, considerable research has focused on β -cell replacement, immune modulation, stem-cell-derived β -cells, gene therapy, β -cell regeneration, and neuroimmune pathways. An interesting emerging concept is that pancreatic function is regulated not only by endocrine and immune mechanisms but also by pancreatic sensory nerves. In particular, transient receptor potential vanilloid-1-positive sensory neurons and the neuropeptide Substance P have been implicated in pancreatic inflammation, β -cell stress, and insulin sensitivity. Razavi and colleagues demonstrated that modulation of this neuronal pathway profoundly altered diabetes development in NOD mice.

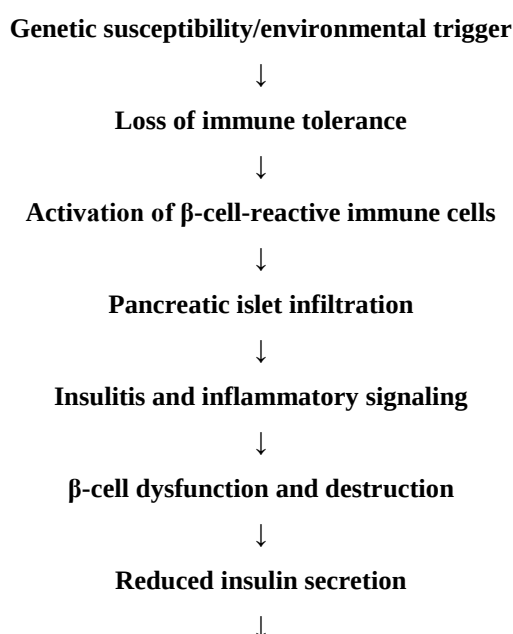
At the same time, preproinsulin gene-transfer experiments have demonstrated that tissues outside the pancreas can experimentally be engineered to synthesize insulin or insulin precursors. Recombinant viral vectors and plasmid systems containing preproinsulin genes have consequently been explored as experimental alternatives for maintaining basal insulin production.

2. PATHOPHYSIOLOGY OF IDDM

The central pathological event in T1DM is destruction of pancreatic β -cells by an inappropriate autoimmune response. Genetic predisposition and environmental influences contribute to the loss of immunological tolerance toward β -cell antigens. Antigen-presenting cells participate in activation of autoreactive lymphocytes, followed by recruitment of immune cells into pancreatic islets.

The resulting inflammatory lesion, known as insulitis, contains T lymphocytes together with macrophages, dendritic cells and other immune components. Continued inflammation progressively reduces functional β -cell mass.

The sequence can be summarized as:



Hyperglycemia



Clinical T1DM

A therapy capable of both preserving remaining β -cells and restoring insulin production could therefore theoretically provide advantages over a strategy addressing either mechanism alone.

3. PREPROINSULIN BIOLOGY

Insulin biosynthesis begins with translation of **preproinsulin**, the initial precursor of insulin.

Preproinsulin consists sequentially of:

Signal peptide $\rightarrow$ B chain $\rightarrow$ C-peptide $\rightarrow$ A chain

The signal sequence directs the newly synthesized molecule into the endoplasmic reticulum. Removal of the signal peptide produces **proinsulin**. Proinsulin subsequently folds and forms the characteristic disulfide bonds connecting the A and B chains.

Within secretory granules, proinsulin is proteolytically processed, producing:

Mature insulin + C-peptide

Efficient intracellular processing is therefore an important consideration in any insulin gene-replacement approach.

4. PREPROINSULIN GENE THERAPY IN EXPERIMENTAL DIABETES

A major concept behind preproinsulin gene therapy is to transform an accessible tissue into a surrogate source of insulin.

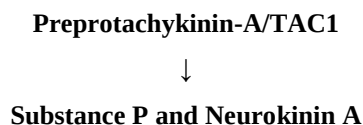
Skeletal muscle has been investigated because it is relatively accessible and capable of long-term expression of introduced genes. In an early NOD-mouse study, recombinant adeno-associated viral delivery of the complete rat preproinsulin II gene resulted in expression of insulin messenger RNA at the treated muscle site and a substantially greater proportion of treated animals remaining euglycemic than control animals during follow-up. Other investigators evaluated plasmid DNA encoding human preproinsulin in streptozotocin-induced diabetic mice. Expression of insulin messenger RNA in skeletal muscle was associated with increased circulating insulin and reduction of hyperglycemia.

Croze and Prud'homme further studied modified, furin-processable preproinsulin constructs. Their results demonstrated that insulin precursor expression could persist for several weeks and that a proportion of the synthesized precursor could be processed into mature insulin. These experiments support the principle that extra-pancreatic insulin production is biologically achievable.

However, an important limitation remains: ordinary skeletal muscle does not reproduce the sophisticated glucose-responsive secretory machinery of pancreatic β -cells. Consequently, constitutive insulin production carries theoretical risks of insufficient insulin during hyperglycemia or excessive insulin during fasting.

5. PREPROTACHYKININ AND SUBSTANCE P

Substance P is an 11-amino-acid neuropeptide belonging to the tachykinin family. The principal precursor gene associated with Substance P is historically called preprotachykinin-A (PPT-A) and is now generally referred to as TAC1. TAC1 processing can produce several tachykinin peptides, particularly:



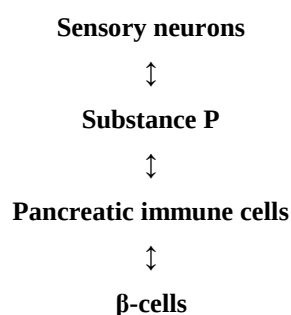
Substance P preferentially activates the **neurokinin-1 receptor (NK1R)**, a G-protein-coupled receptor. Although Substance P was initially investigated primarily as a neurotransmitter involved in pain and neurogenic inflammation, it is now recognized as a broader neuroimmune signaling molecule. Interestingly, preprotachykinin-A expression has been detected transiently in endocrine pancreatic cells during fetal and neonatal pancreatic development. Both insulin-containing and non-insulin-containing endocrine cells can express tachykinin-related peptides during development, whereas expression declines markedly in the adult pancreas. These observations led to the hypothesis that tachykinins may participate in endocrine pancreatic growth and maturation.

6. SUBSTANCE P, TRPV1 AND THE PANCREATIC NEUROIMMUNE AXIS

One of the most important studies connecting Substance P with autoimmune diabetes was reported by Razavi et al. in *Cell*. The investigators demonstrated an important interaction between pancreatic TRPV1-positive sensory neurons, Substance P signaling, pancreatic inflammation, insulin sensitivity and diabetes in the NOD mouse.

The diabetes-associated NOD TRPV1 pathway exhibited altered sensory neuronal function. Manipulation of this pathway changed β -cell stress and inflammatory infiltration of pancreatic islets. Most importantly, pancreas-directed administration of Substance P was associated with reversal of abnormal insulin resistance, marked reduction of insulinitis and temporary reversal of diabetes in a proportion of recently diabetic NOD mice.

These results introduced the concept that T1DM involves an interaction among:



rather than representing exclusively an immune-versus- β -cell disorder.

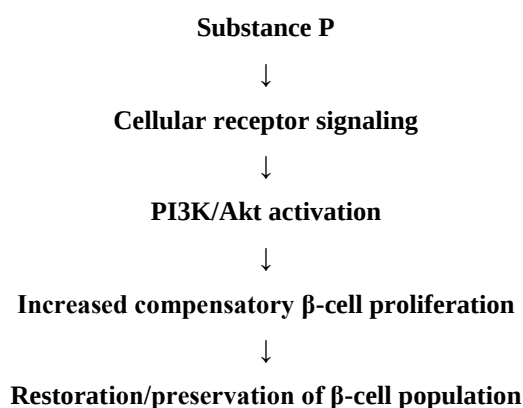
7. NK1 RECEPTORS IN AUTOIMMUNE DIABETES

The biological actions of Substance P are predominantly mediated through NK1 receptors. NK1 receptor expression has been demonstrated in pancreatic islets of NOD mice and particularly in association with immune-cell infiltration. NK1 receptor expression has also been observed on several infiltrating immune-cell populations, including lymphocytes, macrophages and dendritic cells. This finding is important because Substance P may therefore influence both pancreatic endocrine cells and the immune cells responsible for insulinitis.

The Substance P–NK1R system should nevertheless not be described simply as anti-inflammatory. Depending on tissue, receptor distribution, concentration and disease state, Substance P can produce inflammatory as well as immunomodulatory effects. Consequently, the biological context is critical when considering Substance P as a possible therapeutic molecule.

8. SUBSTANCE P AND B-CELL REGENERATION

Jung and colleagues investigated Substance P in streptozotocin-induced pancreatic injury and experimental T1DM. They reported that Substance P enhanced compensatory proliferation of pancreatic β -cells following acute pancreatic injury. Importantly, the investigators found evidence that the proliferative response involved activation of the phosphoinositide 3-kinase/Akt signaling pathway. The proposed sequence is:



The same study showed that chronic systemic Substance P treatment preserved β -cell mass and reduced insulinitis in streptozotocin-induced diabetic mice. Importantly, Substance P did not merely function as a classical insulin secretagogue in these experiments. Its actions appeared to involve alteration of β -cell survival/regeneration and the surrounding tissue environment.

9. SUBSTANCE P IN SPONTANEOUS AUTOIMMUNE DIABETES

The subsequent study by Um and colleagues evaluated Substance P in NOD mice, which provide a spontaneous autoimmune model of T1DM.

Chronic systemic Substance P treatment:

- Delayed development of hyperglycemia,
- Preserved pancreatic β -cell populations,
- Reduced immune-cell infiltration into pancreatic islets, and
- Produced evidence of immune modulation.

These observations are particularly important because the NOD model more closely represents autoimmune β -cell destruction than purely toxin-induced β -cell injury.

10. PUBLISHED ANIMAL EVIDENCE FOR SUBSTANCE P

The important experimental paradigms can be summarized as follows.

Study/model	Substance P strategy	Delivery concept	Major observation
Prediabetic NOD mouse	Acute SP treatment	Pancreas-targeted administration	Major reduction of islet inflammatory infiltration
Newly diabetic NOD mouse	Acute SP treatment	Pancreas-targeted administration	Improvement in insulin sensitivity and temporary reversal of hyperglycemia in responding animals
STZ-induced pancreatic injury	SP treatment	Systemic administration	Increased compensatory β -cell proliferation
STZ-induced T1DM	Chronic SP treatment	Systemic administration	Preservation/restoration of β -cell mass and reduced insulinitis
Spontaneous NOD T1DM	Chronic SP treatment	Systemic administration	Delayed hyperglycemia and reduced immune infiltration

The Razavi study established the importance of pancreas-directed Substance P signaling in NOD diabetes, whereas the later Jung and Um studies demonstrated that prolonged systemic exposure can also influence β -cell preservation and autoimmune inflammation.

These interventions remain experimental animal-model approaches and should not be interpreted as established Substance P treatment regimens for humans.

11. POSSIBLE MECHANISMS OF SUBSTANCE P IN T1DM

The available evidence suggests several complementary mechanisms.

11.1 Reduction of pancreatic inflammation

Substance P can alter immune-cell behavior within pancreatic islets and decrease inflammatory infiltration under specific experimental conditions.

11.2 Improvement of insulin sensitivity

The TRPV1/Substance P pathway appears capable of influencing whole-body and pancreatic insulin sensitivity. Correction of abnormal sensory neuronal signaling in NOD mice was associated with improved metabolic function.

11.3 Preservation of β -cell mass

Chronic Substance P exposure preserved β -cell populations in both chemically induced and spontaneous models of diabetes.

11.4 Stimulation of compensatory β -cell proliferation

Substance P stimulated β -cell proliferation following pancreatic injury, apparently through Akt-associated signaling.

11.5 Neuroimmune modulation

Substance P provides a functional link between pancreatic sensory nerves and immune regulation. The peptide therefore potentially acts upstream of several processes involved in T1DM rather than merely replacing insulin.

12. Preproinsulin Versus Substance P: Complementary Therapeutic Roles

The two approaches address fundamentally different components of diabetes.

Feature	Preproinsulin strategy	Substance P strategy
Principal target	Insulin deficiency	Neuroimmune/ β -cell environment
Primary objective	Produce insulin	Preserve or restore β -cell function
Mechanism	Expression and processing of insulin precursor	NK1R/neuroimmune signaling and β -cell effects
Experimental platform	Gene delivery	Neuropeptide signaling
Effect on autoimmunity	Limited unless combined with another strategy	Potential immunomodulatory effects
Effect on β -cell regeneration	Indirect/limited	Experimental evidence of compensatory proliferation
Major challenge	Glucose-responsive insulin regulation	Short biological lifetime and context-dependent signaling

This distinction is essential.

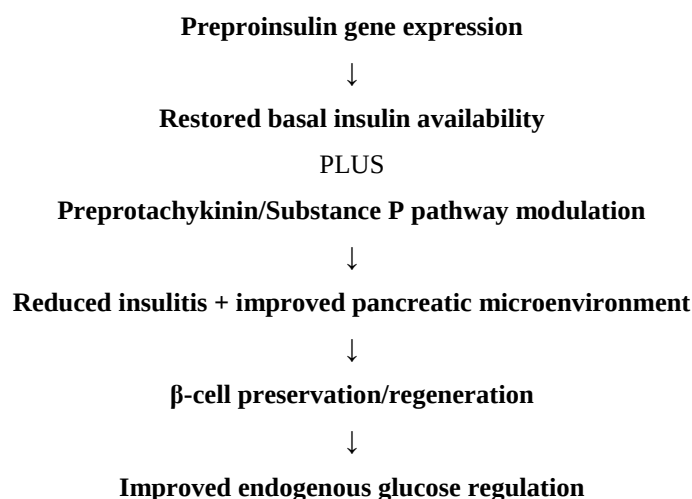
Preproinsulin replaces the missing hormonal output.

Substance P attempts to modify the pathological environment responsible for loss of that output.

13. CONCEPTUAL COMBINED STRATEGY

A scientifically attractive future hypothesis would involve simultaneously addressing insulin deficiency and pancreatic inflammation.

Conceptually:



This represents an attractive research hypothesis, but the available evidence does **not** establish a clinically validated combined preproinsulin–Substance P therapy.

The distinction between an experimentally plausible combination and a proven therapy should therefore be maintained in any review article.

14. SUBSTANCE P AND HUMAN TRANSLATION

Interest in the experimental findings eventually led to registration of a Phase I investigation of Substance P in recent-onset T1DM, designed principally to investigate safety and pharmacological activity using pancreas-directed administration.

The existence of an early clinical investigation demonstrates translational interest but does not establish clinical effectiveness.

Animal-model findings cannot be directly converted into human therapeutic regimens because of differences in:

- pancreatic anatomy,
- immune-cell populations,
- peptide pharmacokinetics,
- receptor expression,
- residual β -cell mass,

Substance P therefore remains an investigational concept rather than an accepted replacement for insulin therapy.

15. ADVANTAGES OF THE PROPOSED THERAPEUTIC CONCEPT

Several theoretical advantages can be identified.

First, preproinsulin-based approaches may provide prolonged insulin biosynthesis rather than requiring repeated administration of insulin. Second, Substance P addresses biological mechanisms upstream of insulin deficiency, particularly β -cell stress and neuroimmune inflammation.

Third, β -cell preservation shortly after T1DM diagnosis could potentially retain endogenous C-peptide production. Fourth, simultaneously targeting metabolic deficiency and autoimmune tissue injury could theoretically produce better results than either intervention individually. Finally, understanding the neuroimmune regulation of the pancreatic islet may reveal additional therapeutic targets beyond Substance P itself.

16. FUTURE RESEARCH DIRECTIONS

Future investigations should concentrate on glucose-responsive regulation of preproinsulin expression rather than continuous constitutive insulin production. Researchers should also determine which pancreatic and immune-cell populations mediate the beneficial effects attributed to Substance P and define how NK1R signaling changes across different stages of T1DM.

Another important objective will be identifying the therapeutic window during which sufficient viable β -cell mass remains for regenerative or protective therapy to be effective. Future work may also investigate engineered peptide-delivery systems, tissue-specific expression systems, β -cell-targeted vectors, combination immunotherapy and regulated insulin gene expression.

Finally, human studies will be required to determine whether the striking neuroimmune effects observed in NOD mice are reproducible in patients with recent-onset T1DM.

17. CONCLUSION

Preproinsulin and preprotachykinin/Substance P represent two intriguing but fundamentally different experimental approaches to the management of insulin-dependent diabetes mellitus. Preproinsulin gene therapy attempts to compensate for insulin deficiency by establishing alternative cellular sources of insulin. Animal experiments have demonstrated prolonged insulin precursor expression and improvement in hyperglycemia following gene transfer. Substance P, in contrast, targets the complex interaction among sensory neurons, pancreatic β -cells and immune cells. Experimental studies demonstrate that Substance P can reduce insulinitis, improve insulin sensitivity, promote compensatory β -cell proliferation, preserve β -cell mass and delay diabetes progression in selected mouse models.

The most interesting long-term concept is therefore not simply insulin replacement but a dual approach combining metabolic restoration with protection of the pancreatic neuroimmune microenvironment. Nevertheless, evidence available at present does not support describing preproinsulin plus Substance P as an established treatment for T1DM. Both approaches remain investigational, and insulin replacement continues to be essential therapy for established type 1 diabetes.

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