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Therapeutic potential of α7 nicotinic acetylcholine receptor agonists to combat obesity, diabetes, and inflammation

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Abstract

The cholinergic anti-inflammatory reflex (CAIR) represents an important homeostatic regulatory mechanism for sensing and controlling the body's response to inflammatory stimuli. Vagovagal reflexes are an integral component of CAIR whose anti-inflammatory effects are mediated by acetylcholine (ACh) acting at α7 nicotinic acetylcholine receptors (α7nAChR) located on cells of the immune system. Recently, it is appreciated that CAIR and α7nAChR also participate in the control of metabolic homeostasis. This has led to the understanding that defective vagovagal reflex circuitry underlying CAIR might explain the coexistence of obesity, diabetes, and inflammation in the metabolic syndrome. Thus, there is renewed interest in the α7nAChR that mediates CAIR, particularly from the standpoint of therapeutics. Of special note is the recent finding that α7nAChR agonist GTS-21 acts at L-cells of the distal intestine to stimulate the release of two glucoregulatory and anorexigenic hormones: glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). Furthermore, α7nAChR agonist PNU 282987 exerts trophic factor-like actions to support pancreatic β-cell survival under conditions of stress resembling diabetes. This review provides an overview of α7nAChR function as it pertains to CAIR, vagovagal reflexes, and metabolic homeostasis. We also consider the possible usefulness of α7nAChR agonists for treatment of obesity, diabetes, and inflammation.

Keywords α7nAChR · Cholinergic anti-inflammatory reflex · Obesity · Diabetes · GLP-1 · PYY

Abbreviations

α7nAChR	α7 nicotinic acetylcholine receptor
ACh	acetylcholine
AChE	acetylcholinesterase
ANS	autonomic nervous system
ATM	adipose tissue macrophage
BCL2	B cell lymphoma 2
BCM	beta-cell mass
CAIR	cholinergic anti-inflammatory reflex
CNS	central nervous system
CREB	cAMP response element-binding protein
DIO	diet-induced obesity
DMV	dorsal motor nucleus of the vagus
EEC	enteroendocrine cell
ENS	enteric nervous system
ER	endoplasmic reticulum
GABA	gama aminobutyric acid
GPCR	G protein-coupled receptor
GLP-1	glucagon-like peptide-1
GTS-21	3-(2,4-dimethoxy-benzylidene)anabaseine
HbA1c	glycated hemoglobin 1c
HFD	high fat diet
IFN	interferon
IgG Fc	immunoglobulin G fragment crystallizable
IκBα	inhibitor of nuclear factor kappa B alpha
IL	interleukin
IRE1α	inositol-requiring enzyme 1α
KO	knockout
JAK2	Janus kinase 2
JNK	c-Jun N-terminal kinase
LPS	lipopolysaccharide
MCP-1	chemokine monocyte chemoattractant protein-1

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MLDS	multiple low-dose streptozotocin
MOMP	mitochondrial outer membrane permeabilization
MyD88	myeloid differentiation factor 88
mTOR	mammalian target of rapamycin
NAFLD	non-alcoholic fatty liver disease
NF-κB	nuclear factor kappa B
NOS	nictric oxide synthase
NPY	neuropeptide Y
NPY2R	neuropeptide Y2 receptor
NTS	nucleus tractus solitarius
PAM	positive allosteric modulator
p70S6K	ribosomal protein S6 kinase beta-1
PC1/3	prohormone convertase 1/3
PI3K	phosphatidylinositol 3-kinase
PKA	protein kinase A
PKB	protein kinase B
PNU 282987	*N*-(3*R*)-1-Azabicyclo[2.2.2] oct-3-yl-4-chlorobenzamide
POMC	proopiomelanocortin
PYY	peptide YY
RNase	endoribonuclease
SAT	subcutaneous adipose tissue
siRNA	small interfering RNA
STAT3	signal transducer and activator of transcription 3
STZ	streptozotocin
XBP1s	spliced X-box binding protein
T1D	type 1 diabetes
T2D	type 2 diabetes
TGF	transforming growth factor
TXNIP	thioredoxin-interacting protein
TLR4	Toll-like receptor-4
TNF	tumor necrosis factor
UCD-T2D	UC Davis type 2 diabetes model rat
UPR	unfolded protein response
VAT	visceral adipose tissue
VIP	vasoactive intestinal polypeptide
VN	vagus nerve
ZDF	Zucker diabetic fatty rat

1 Introduction

The pathogenesis of obesity and type 2 diabetes (T2D) are intertwined in that these two disorders participate in a metabolic syndrome in which abnormalities of appetite control, body weight, energy expenditure, glycemia, and lipid metabolism exist in combination with elevated blood pressure and low-grade systemic inflammation [1]. Here, we review the potential for a new α7 nicotinic acetylcholine receptor (α7nAChR) agonist therapy to treat the metabolic syndrome, a concept based on published data (see Table 1). The approach exploits the cholinergic anti-inflammatory reflex (CAIR) that is mediated by the vagus nerve (VN; Cranial Nerve X). In this circuit (Fig. 1), pro-inflammatory cytokines stimulate VN sensory afferents to initiate a vagovagal reflex conveyed through the central nervous system (CNS), ultimately leading to acetylcholine (ACh) release from parasympathetic VN efferents of the autonomic nervous system (ANS) [16]. As established for CAIR, this ACh then activates the α7nAChR on immune system cells to suppress cytokine production and to produce an anti-inflammatory effect [17]. Here we propose that defects of the vagovagal reflex underlying CAIR explain, at least in part, why obesity, T2D, and inflammation coexist in the metabolic syndrome [18, 19]. This concept is consistent with the established ability of vagovagal reflexes to control appetite, body weight, energy expenditure, and glycemia [20, 21]. Thus, there is renewed interest in the α7nAChR that mediates CAIR, particularly from the standpoint of therapeutics and drug discovery. This review provides an overview of α7nAChR expression and function as it pertains to CAIR, vagovagal reflexes, and metabolic homeostasis. We also consider the possible usefulness of α7nAChR agonists for treatment of obesity, diabetes, and inflammation.

2 Role of CAIR and α7nAChR in adaptation to inflammation

2.1 Overview

Infection, tissue trauma, and other disease states such as arthritis and inflammatory bowel disease cause local and/or systemic inflammation. When inflammation is uncontrolled or excessive, tissue damage results from uncontrolled release of pro-inflammatory cytokines (TNF, IL-1), histones, high-mobility group box 1 protein, mitochondrial DNA, and lipopolysaccharide (LPS) [22]. Under these conditions, the activation and release of inflammatory substances from white blood cells and other tissues triggers homeostatic self-protective processes that are mediated by CAIR so as to prevent further tissue damage [16, 17] (Fig. 1). CAIR initiates ACh release from VN efferents, thereby stimulating the α7nAChR on immune system cells to suppress pro-inflammatory cytokine production [23]. Simultaneously, CAIR, acting through the α7nAChR, stimulates production and release of anti-inflammatory substances (IL-4, IL-10, TGFβ) [23].

Initiation of CAIR requires a vagovagal reflex in which afferent input to the brainstem nucleus tractus solitarius (NTS) stimulates efferent output originating from the dorsal motor nucleus of the vagus (DMV) [16, 17] (Fig. 1). CAIR targets lymphocytes, macrophages, dendritic cells, adipocytes, keratinocytes, endothelial cells, and epithelial cells of the intestine and lung to suppress inflammation [24–26].

Table 1 *In vivo* and *in vitro* tissue-specific effects of α7nAChR agonists

Tissue/Cell/Organism	Agonist	Effect	Reference
Adipocyte (3T3-L1)	GTS-21	Anti-Inflammatory Inhibits NF-κB	[2]
Adipocyte (human)	PNU 282987	Anti-Inflammatory	[3]
Adipocyte (mouse)	PNU 282987	STAT3 activation	[4]
Adipocyte (mouse, human)	ICH3	Anti-Inflammatory Enhanced viability	[5]
Muscle (mouse, C2C12)	PNU 282987	STAT3 activation Glucose uptake	[4]
Skeletal Muscle (DIO mouse)	ICH3	Improved insulin sensitivity	[5]
Vascular smooth muscle (Rat)	Nicotine GTS-21	Insulin signaling p44/42 MAPK α7nAChR expression	[6]
Liver (Kupffer cells)	Nicotine	Protects against ConA-induced hepatitis	[7]
Liver (Kupffer cells)	Nicotine PNU 282987	Protects against Fas-induced hepatocyte apoptosis	[8]
CNS (mouse hypothalamus)	PNU 282987	Anorexic JAK2/STAT3 activation	[9]
Islets of Langerhans (mouse)	PNU 282987	Anti-Inflammatory Anti-apoptosis	[10]
Enteroendocrine (L-cells)	GTS-21	Enhanced viability GLP-1 secretion	[11]
Keratinocyte (human)	AR-R17779 Tropisetron	Anti-Inflammatory Suppress TNFα	[12]
db/db obese mouse	TC-7020	Decreased weight, food intake. Reduced blood glucose, triglycerides, TNFα.	[13]
db/db, DIO mouse	Nicotine	Improved glucose homeostasis, insulin sensitivity.	[14]
Zucker fatty rat	Nicotine	Improved glucose homeostasis, insulin sensitivity.	[15]

Importantly, the neural circuitry of CAIR may not be exclusively engaged by pro-inflammatory cytokines since vagovagal reflexes are also stimulated by metabolites, nutrients, and intestinal hormones [27]. Since vagovagal reflexes play an important role in the control of metabolic homeostasis [18, 20, 21], the neural circuitry of CAIR is well situated to participate in the dual control of inflammation and whole-body metabolism in the healthy and disease states.

2.2 Physiological and clinical relevance of CAIR

The VN afferents that mediate CAIR express receptors for multiple inflammatory molecules including IL-1, TNF, IgG FC, LPS, and prostaglandins [28]. For example, stimulation of these afferents after intraperitoneal administration of IL-1 or LPS activates CAIR to counteract the febrile and acute phase responses of inflammation [29]. Subdiaphragmatic vagotomy abrogates the anti-inflammatory effects of CAIR [28], whereas electrical stimulation of the VN produces an anti-inflammatory effect analogous to CAIR. Vagal nerve stimulation (VNS) ameliorates the inflammatory action of intravenously administered LPS, while also reducing hepatic TNF synthesis, lowering serum levels of TNF, and suppressing shock [30]. Thus, CAIR provides a rapid anti-inflammatory reflex to counteract uncontrolled release of pro-inflammatory cytokines.

Multiple neural networks of CAIR exist, not all of which require VN release of ACh acting directly at α7nAChR on immune system cells. For example, in the spleen, a vagosympathetic reflex exists in which ACh released from VN efferents stimulates the splenic nerve to initiate norepinephrine release, which in turn stimulates ACh release from a subset of T cells that express the ACh biosynthetic enzyme choline acetyltransferase [31] (Fig. 1). Resultant stimulation of α7nAChR on adjacent splenic macrophages suppresses TNF production to reduce inflammation [31]. On the other hand, in the gastrointestinal tract, VN efferents innervate nNOS-VIP-ACh positive enteric neurons to stimulate ACh release, which then acts at intestinal resident macrophages to exert an α7nAChR-mediated effect to inhibit TNF production and inflammation [32, 33]. VN efferents participating in CAIR also innervate the liver, and their stimulation attenuates inflammation under conditions of LPS-induced sepsis, hepatitis, and ischemia-reperfusion [34]. These hepatic VN efferents activate α7nAChR to reduce Fas-induced apoptosis of hepatocytes, most likely through an effect on Kupffer cells to reduce reactive oxygen species production [8]. Nicotine activation of α7nAChR in Kupffer cells is also reported to

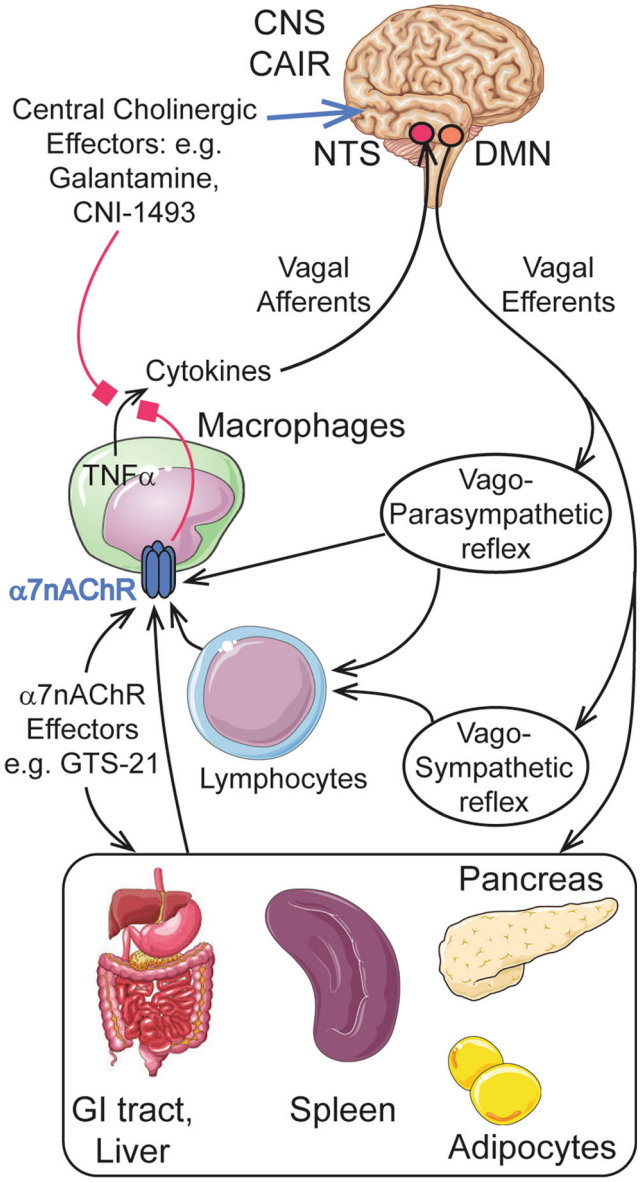

Fig. 1 Neurocircuitry of vagovagal reflexes underlying CAIR and mediated by α7nAChR with consequent engagement of multiple organ systems including the central nervous system (CNS) brainstem nucleus tractus solitarius (NTS) and the dorsal motor nucleus (DMN) of the vagus nerve so that TNFα proinflammatory cytokine production is inhibited in cells of the immune system. Black arrows indicate stimulatory effects, whereas red boxes indicate inhibitory effects

reduce concanavalin A-induced hepatitis through the inhibition of NF-κB [7].

When considering the anti-inflammatory effects of CAIR summarized above, it is important to note that *in vivo* studies using α7nAChR knockout (KO) mice demonstrate that this receptor mediates the anti-inflammatory effects of VNS under conditions of LPS administration [25]. This finding is consistent with the identification of α7nAChR as the target of nicotine in assays monitoring suppression of cytokine release in LPS-treated macrophages [35]. VNS that emulates CAIR also influences food intake, body weight, fat mass, glycemia, and insulin sensitivity [27, 36–46]. For example, VNS in Zucker diabetic fatty (ZDF) rats improves glycemic control, lowers levels of glycated hemoglobin (HbA1c), and improves insulin sensitivity in muscle and liver [42]. In obese mini-pigs, VNS decreases body weight and subcutaneous fat mass, and is coupled to improved insulin sensitivity, and improved brain and skeletal muscle glucose uptake [43]. Furthermore, rats with diet-induced obesity (DIO) also demonstrate reductions in food intake and body weight in response to VNS [40]. What relevance such findings have to humans is not yet certain, and in fact a block of VN conduction is reported to lower body weight in humans [47, 48]. Still, these findings obtained in multiple models do demonstrate that VNS leads to significant metabolic alterations consistent with a major role for CAIR in metabolic homeostasis.

From a medical systems physiology standpoint, it is noteworthy that subcutaneous mature adipocytes from obese human subjects display reduced α7nAChR expression, and that this anomaly is reversible after dieting to achieve significant weight loss [3]. It is further demonstrated that α7nAChR activation induces an anti-inflammatory effect in human adipocytes that is potentially beneficial so as to ameliorate this component of the metabolic syndrome [3]. These human studies compliment findings obtained using leptin receptor-deficient obese mice (*db/db*) or DIO mice where CAIR activation improves insulin sensitivity and insulin-sensitive glucose uptake in adipose tissue [14], and also in liver and muscle [4]. Thus, complex inter-organ communication is under the control of vagovagal reflexes that mediate CAIR, and that are also of major relevance to the control of metabolic homeostasis [49]. Dysfunctions of this inter-organ communication are likely to be important contributing factors to the metabolic syndrome.

Finally, is especially interesting that VNS increases circulating levels of glucagon-like peptide-1 (GLP-1), an intestinal "incretin" hormone that lowers levels of blood glucose, suppresses appetite, delays gastric emptying, improves cardiovascular function, and counteracts inflammation [50–52]. This action of VNS is mediated by VN efferents that innervate the intestinal wall where enteroendocrine L-cells that release GLP-1 are located [52]. These L-cells also release Peptide YY (PYY), a hormone that suppresses appetite [52]. Thus, an attractive hypothesis is that the afferent and efferent components of vagovagal reflexes mediating CAIR overlap functionally and anatomically with vagovagal reflex circuitry controlling whole-body metabolism and endocrine system function.

2.3 Therapeutic approaches targeting vagovagal reflex circuitry

It is increasingly recognized that inflammation and metabolic decompensation in the disease state result from a loss of

function in afferent and/or efferent limbs of vagovagal reflex circuitry [21]. For example, consumption of a high fat diet (HFD) results in VN dysfunction accompanied by obesity, hyperglycemia, and adipose tissue inflammation in rodents and humans [53, 54]. Mechanistically, chronic ingestion of the HFD results in a decreased sensitivity of VN afferents to metabolites, nutrients, and hormones that modulate vagovagal reflex function through their direct effects at the peripheral terminations of these sensory neurons [55, 56]. For example, the stimulation of VN afferent activity by intestinally absorbed glucose is diminished in the UCD-T2D rat model of human T2D in which there is adult-onset obesity, hyperglycemia, and adipose tissue inflammation [57].

Collectively, these findings suggest that new therapies designed to reverse or compensate for a loss of vagovagal reflex function might constitute novel therapeutic options when considering how to combat obesity, diabetes, and inflammation. In this regard, VNS might serve as one method by which to override defective vagovagal reflex function. However, a pharmacological approach based on the use of medications that emulate CAIR is also conceivable. Investigational agents include the anti-cytokine CNI-1493 and the acetylcholinesterase (AChE) galantamine that acts within the CNS at muscarinic ACh receptors to stimulate VN efferent activity emulating CAIR [58] (Fig. 1). They also include α7nAChR agonists that were previously investigated for use in the treatment of inflammatory, neurodegenerative, and psychiatric disorders [59–62]. In the following sections we discuss α7nAChR agonists that serve as lead compounds for treatment of obesity, diabetes, and inflammation.

3 Molecular, pharmacological, and signaling properties of α7nAChR

When reviewing the potential usefulness of α7nAChR agonists as therapeutic agents, it is first necessary to briefly summarize available information concerning their molecular target of action, the α7nAChR [63–66]. It is one member of the nAChR family, the first member of which was cloned from the electric organ of the *Torpedo* ray [67]. All such nACh receptors are comprised of pentameric assemblies derived from a gene pool of 17 available α, β, γ, δ, and ε protein subunits that co-assemble to form ligand-gated cation channels [68]. Besides their classic role in nicotinic cholinergic transmission at the motor neuron end plate on skeletal muscle, multiple isoforms of nAChR are expressed in numerous non-neuronal cell types throughout the body [68]. Unlike most other isoforms of nAChR that are solely heteropentameric in structure, the α7nAChR is formed as either a homopentameric channel comprised solely of α subunits, or as a heteropentamer of α7β2 subunits in which two α7 subunits must be present for functionality [69, 70].

The tissue-specific subunit composition of each isoform of nAChR determines its functional and pharmacological properties [71]. One feature of α7nAChR is that it has high selectivity and permeability for Ca^{2+} in comparison to other nAChRs [72]. Thus, α7nAChR agonists exert Ca^{2+}-mediated actions to regulate signaling pathways and gene expression networks [73, 74] (Fig. 2). Ca^{2+} entry through α7nAChR can also initiate Ca^{2+}-induced Ca^{2+} release from intracellular Ca^{2+} stores, thereby generating an additional increase of cytosolic [Ca^{2+}] that amplifies this effect [75]. Such ionotropic properties of α7nAChR are complemented by a metabotropic signaling function in which it interacts with G_q heterotrimeric G proteins to stimulate phospholipase C, generate IP_3, and further mobilize intracellular Ca^{2+} [76].

α7nAChR opens in response to low concentrations of nicotine, but then rapidly desensitizes [66]. These properties contrast with the α3β4 nAChR in autonomic ganglia that is insensitive to low concentrations of nicotine, and that is slow to desensitize [77]. Since α7nAChR is not expressed at high levels in autonomic ganglia [78, 79], α7nAChR agonists are less likely to exert undesirable ANS side effects related to direct ganglionic stimulation. Investigations of α7nAChR function are facilitated by the availability of mice with a KO of *CHRNA7*, the gene that codes for α7nAChR [80]. Furthermore, an armamentarium of α7nAChR agonists and

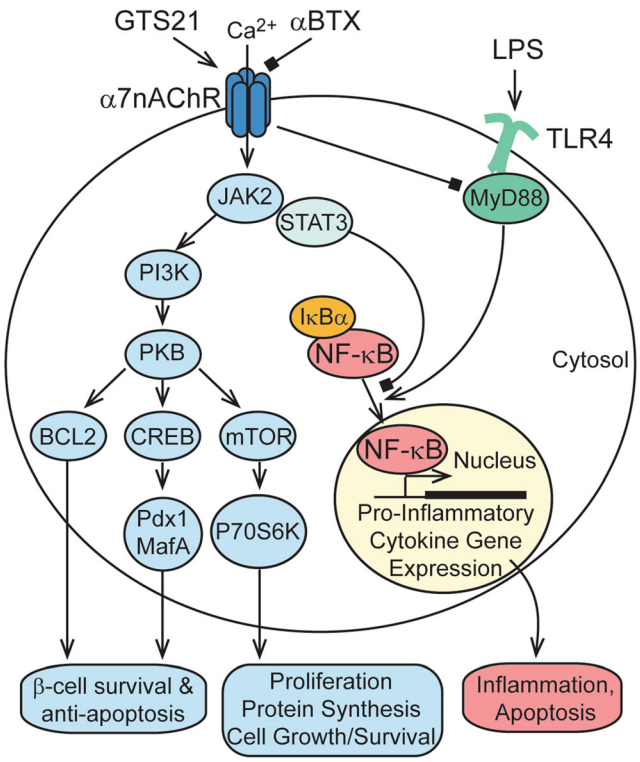

Fig. 2 Growth factor-like signal transduction pathways under the control of α7nAChR and relevant to CAIR, proinflammatory cytokine production, cell growth/survival, inflammation, apoptosis, and pancreatic β-cell survival. For additional details concerning α7nAChR and β-cell survival, see Gupta et al. [10]

antagonists is available [81–84]. Selective partial or full agonists for stimulation of α7nAChR include choline, GTS-21, PNU 282987, PHA 568487 and ICH3 (see Table 2). Compliments to conventional agonists are the "silent" agonists, such as m-bromo PEP and NS 6740, that induce receptor desensitization with little or no channel opening, but that still play a role in regulating inflammation [92, 94]. Positive allosteric modulators (PAMs) that enhance agonist action at α7nAChR include 5-hydroxyindole (5HI), PNU 120596, A 867744, and B 973B. PAMs fall into two categories. Type-1 PAMs such as 5HI simply increase agonist-evoked current amplitude, whereas type II PAMs such as PNU 120596 increase current amplitude and also reactivate desensitized receptors [95]. Available α7nAChR antagonists include α-bungarotoxin and methyllcaconitine. The selectivities of these compounds are not absolute, and in fact GTS-21 exhibits off-target antagonist actions at serotonin type-3 receptors (5-HT_3R) when tested at high concentrations [96]. Interestingly, the *CHRNA7* gene underwent a partial duplication to produce *CHRFAM7A* [97, 98]. Its gene product dupα7 acts as a dominant negative regulator of human α7nAChR [99]. Thus, dupα7 has the potential to modulate CAIR [100], but it is not discussed in detail here.

α7nAChR agonists also regulate JAK2, STAT3, PI3K/PKB, BCL2, CREB, mTOR, p70S6K, and NF-κB signaling pathways [26, 101–103] (Fig. 2). In the absence of inflammatory stimuli, NF-κB subunits RelA and p50 are located in the cytoplasm in association with the inhibitory protein IκBα. Inflammatory mediator such as LPS activate Toll-like receptors (TLR4) in association with their MyD88 adaptor proteins, which triggers the phosphorylation and degradation of IκBα. This releases free NF-κB subunits so that they can translocate to the nucleus, bind to specific DNA regulatory elements, and stimulate pro-inflammatory gene expression (Fig. 2). α7nAChR agonists inhibit this inflammatory response by suppressing NF-κB nuclear translocation [26, 101–103].

Table 2 Representative α7nAChR agonists and EC_{50} values for α7nAChR stimulation

Agonist	EC_{50} Rat (nM)	EC_{50} Human (nM)	Reference
GTS-21	310	2300	[85]
4-OH-GTS-21	170	450	[85]
PNU 282987	27		[86]
PHA 543613	8.8		[87]
PHA 709829	3.4		[88]
ICH3	4.6	48.7	[5, 89–91]
NS 6784	3.3		[92]
NS 6780	1.1		[92]
BMS 933043	3.3	8.1	[93]

4 α7nAChR regulation of inflammation in obesity

Obesity is a key risk factor for metabolic abnormalities including insulin resistance and T2D, as wells as atherosclerosis and nonalcoholic fatty liver disease (NAFLD) [104]. Furthermore, obesity and inflammation are linked with respect to their mutual contributions to the metabolic syndrome [1]. Thus, it is significant that α7nAChR agonists act in animal models of obesity to suppress inflammation and to improve insulin sensitivity in liver, muscle and adipose tissue [105–110]. When considering α7nAChR agonist action in the context of obesity, it is useful to summarize relevant features of adipocyte biology. In this regard, adipose tissue was at one time considered to be a metabolically inactive reservoir of stored excess calories. However, it is now recognized that adipocytes secrete metabolites (free fatty acids, glycerol), hormones (leptin, adiponectin, resistin), and inflammatory mediators (TNF and IL-6) [111]. A balance between pro- and anti-inflammatory actions of these secretory products determines the progression or regression of adipose tissue inflammation.

α7nAChR agonists exert their beneficial effects in adipocytes by down regulating the activities of pro-inflammatory mediators, while simultaneously upregulating the activities of anti-inflammatory mediators [16, 17]. Importantly, α7nAChR agonists also counteract inflammatory monocyte infiltration in adipose tissue, a phenomenon under the control of the chemokine monocyte chemoattractant protein-1 (MCP-1) [112]. Similarly, α7nAChR agonists counteract the accumulation and activation of adipose tissue macrophages (ATMs) within fat [14]. This is significant because activated ATMs with the (M1) phenotype secrete inflammatory cytokines (TNF, IL-1, IL-6) that trigger inflammation via the IκB kinase β and c-Jun N-terminal kinase (JNK) pathways [104–107].

Some interesting observations concerning human adipocyte heterogeneity are revealed in studies using the α7nAChR agonist ICH3 [5]. When human adipocytes from obese donors are studied *in vitro*, ICH3 differentially affects IL6 and adiponectin gene expression in visceral adipose tissue (VAT) as compared to subcutaneous adipose tissue (SAT) [5]. This is a gene-specific effect because VAT and SAT show no change in TNFα or leptin expression in response to ICH3 [5]. Such differential actions of ICH3 in VAT and SAT are understandable if there exists fat depot-specific actions of α7nAChR agonists. VAT and SAT have different developmental origins, and it is VAT that is associated with metabolic dysfunction, whereas SAT tends to be protective [113]. Thus, α7nAChR agonist action in VAT might be especially relevant when considering how α7nAChR stimulation reduces excess adiposity. A similar fat depot-specific action of ICH3 is also observed for DIO mice where epididymal fat mass is reduced

by ICH3, whereas perirenal and subcutaneous fat mass are not affected [5]. In these same mice, ICH3 improves insulin sensitivity and glucose tolerance [5].

5 α7nAchR regulation of inflammation in diabetes

We now consider what role α7nAChR plays in the control of inflammation in the context of diabetes, as inferred from studies of α7nAChR agonist action in animal models of hyperglycemia. Leptin receptor-deficient *db/db* mice are one such model in which hyperglycemia, obesity, and low-grade systemic inflammation coexist. Administration of α7nAChR agonist TC-7020 to these *db/db* mice leads to reduced levels of circulating pro-inflammatory TNFα, and also reduced levels of blood glucose and triglycerides [13]. Such effects of TC-7020 are blocked by α7nAChR antagonist methyllycaconitine or by JAK2 inhibitor AG-490, as expected if the α7nAChR-mediated anti-inflammatory action of TC-7020 is linked to improved glucoregulation [13]. Consistent with these findings, treatment with the broad-spectrum nAChR agonist nicotine improves insulin sensitivity and glucoregulation in *db/db* mice [14]. This is also the case for a mouse model of DIO and hyperglycemia resulting from a HFD [4, 114]. Conversely, mice with a KO of α7nAChR exhibit insulin resistance, impaired glucose tolerance, and a reduced ability of nicotine to suppress pro-inflammatory cytokine production [14]. Findings are also reported for Sprague-Dawley rats in which nicotine treatment improves insulin sensitivity and glucoregulation [4]. This ability of nicotine to improve insulin sensitivity is reproduced by α7nAChR agonist PNU 282987, and is lost in α7nAChR KO mice [4]. Such findings are in general agreement with one report that the appearance of hepatic insulin resistance, steatosis, and inflammatory macrophage infiltration is accelerated in α7nAChR KO mice fed the HFD [115]. In contrast, wild-type mice fed the HFD exhibit reduced steatosis and inflammatory infiltration when treated with α7nAChR agonist PNU 282987 [115].

It is interesting that when maternal pregnant mice are fed the HFD, the male offspring of these mice exhibit reduced hepatic α7nAChR expression that is accompanied by insulin resistance [116]. These abnormalities correlate with elevated levels of TNFα and a reduced ability of insulin to stimulate phosphorylation of PKB in hepatocytes of the male offspring [116]. Such findings are understandable if HFD-induced fetal metabolic "programming" during gestation leads to downregulation of hepatic α7nAChR expression, with consequent insulin resistance leading to metabolic decompensation in progeny mice [116, 117].

Another interesting aspect of this story concerns galantamine, an AChE inhibitor that has CNS penetrance and that has been studied in patients with metabolic syndrome [118, 119]. Galantamine slows metabolic inactivation of ACh, thereby increasing the availability of ACh for synaptic transmission mediated by all isoforms of ACh receptors [120]. Surprisingly, galantamine also binds to the nAChR to allosterically enhance its activation by ACh both pre- and post- synaptically [121, 122]. In mice fed the HFD, galantamine decreases plasma levels of IL-6, leptin, MCP-1, and resistin, while increasing levels of adiponectin [123]. Furthermore, galantamine lowers plasma glucose, insulin, homeostatic model assessment of insulin resistance score, and hepatic steatosis [123]. One clinical trial testing galantamine in patients with metabolic syndrome demonstrates reductions in plasma TNFα and leptin, and increased levels of adiponectin and IL-10 [119]. Galantamine also decreases plasma glucose, insulin resistance, and heart rate variability in patients with metabolic syndrome [119]. In summary, these studies demonstrate the ability of α7nAChR agonists and galantamine to improve glycemic control and insulin sensitivity, in part through modulation of adipose tissue inflammation in experimental models of diabetes.

6 α7nAChR regulation of appetite and body weight

Our understanding of α7nAChR agonist action that is relevant to appetite and body weight control is aided by prior studies concerning actions of nicotine in adipose tissue or the CNS. From a systems physiology standpoint, nicotine reduces appetite and food intake while also increasing energy expenditure so that body weight is reduced [124]. When considering the peripheral action of nicotine, it is established that in rodents, a nicotine-stimulated reduction of body weight is proportional to reduced fat mass and an anti-inflammatory effect [125]. For example, in the *fa/fa* leptin receptor-deficient Zucker rat model of obesity, nicotine reduces body weight, lowers levels of TNFα in visceral fat tissues, and reduces fasting levels of blood glucose, while also improving glucose tolerance [15]. To what extent α7nAChR participates in such effects of nicotine is uncertain, especially from the standpoint of its peripheral expression in human adipose tissue. For example, in studies of fat depot SAT isolated from obese human donors not administered nicotine, levels of α7nAChR mRNA and protein are decreased [3]. This finding raises questions concerning whether or not sufficient numbers of α7nAChR exist in SAT of obese subjects in order for nicotine to reduce fat mass. Still, the α7nAChR agonist PNU 282987 produces an anti-inflammatory effect in these same samples of SAT [3]. Furthermore, in SAT samples obtained from obese human donors after a 3-month weight loss intervention, increased levels of α7nAChR levels are detectable [3]. Potentially, pharmacological administration of an α7nAChR agonist targeting both SAT and VAT (as discussed above) might be an effective

strategy to reduce adipose inflammation, increase adipocyte energy expenditure, and reduce fat mass in patients with obesity.

Nicotine also exerts direct stimulatory actions within the CNS to suppress appetite, reduce food intake, increase energy expenditure, and reduce body weight [126]. α7nAChR is expressed at high levels in the hypothalamus [126], and its stimulation by nicotine influences proopiomelanocortin (POMC), neuropeptide Y (NPY), melanin-concentrating hormone, GABA, glutamate, dopamine, and serotonin neurotransmitter circuitry relevant to the control of appetite, food intake, energy expenditure, and body weight [126]. Still, formal proof that α7nAChR does in fact mediate these multiple actions of nicotine within the CNS will require cell type-specific knockouts of α7nAChR gene expression in discreet populations of neurons.

It is remarkable that nicotine activates GLP-1 positive neurons in the NTS, an effect associated with reduced nicotine self-administration in mice [127]. This constitutes a negative feedback "satiety" sensor for nicotine, and it involves GLP-1 acting as a neurotransmitter to excite medial habenular projections to the interpeduncular nucleus so that nicotine reward is decreased [127]. An intriguing possibility is that α7nAChR mediates stimulatory effects of nicotine at GLP-1 positive NTS neurons. If so, VN afferent input to the NTS might activate brainstem neural circuits that stimulate ACh release which then signals through α7nAChR to stimulate GLP-1 release from these same GLP-1 positive neurons. As summarized below, efferent VN input to the intestinal wall may also drive GLP-1 release from L-cells in a α7nAChR-mediated manner. Thus, a situation may exist in which VN efferents release ACh to stimulate L-cell GLP-1 release, thereby allowing GLP-1 to excite VN afferents that project to the NTS, and that in turn stimulate GLP-1 release from NTS neurons. Potentially, coordinate gene expression underlies this neural circuitry in which α7nAChR biosynthesis is linked to GLP-1 biosynthesis in the NTS and L-cells.

Finally, α7nAChR agonists reduce food intake and weight gain in mouse models of DIO [5, 114]. Thus, clinical testing in humans seems warranted in order to evaluate whether α7nAChR agonists have sufficient efficacy to achieve body weight reduction. Potentially, α7nAChR agonists might serve as an add-on therapy in combination with newer approaches to pharmacological weight reduction. For example, liraglutide is a synthetic GLP-1 receptor agonist that emulates the appetite suppressing and blood glucose-lowering actions of incretin hormone GLP-1 [128–130]. Just as impressive, combined administration of naltrexone and bupropion is an effective therapy for weight reduction in obese and diabetic subjects [128, 131, 132]. Still, the adverse side effect of nausea is a frequent complicating factor when administering appetite-suppressant drugs that have strong CNS penetrance [133, 134]. Whether this is also the case for α7nAChR agonists administered to obese subjects is not yet reported.

7 α7nAChR regulation of enteroendocrine L-cell function and viability

Enteroendocrine cells (EEC) are specialized epithelial cells that line the wall of the intestine and that release hormones into the systemic circulation in response to nutrients (e.g., glucose, lipids, amino acids) present within the intestinal lumen after a meal [135]. Specialized microvilli on the apical (i.e., luminal) membrane of the EEC act as nutrient sensors by virtue of their expression of multiple subtypes of nutrient transporters (e.g., Na^+-dependent glucose or amino acid transporters) [136]. These microvilli also express nutrient responsive G protein-coupled receptors (GPCRs) that include the T1R2 amino acid taste receptor, the GPR120 fatty acid receptor, and the TGR5 bile acid receptor [137]. Nutrients acting at the apical membrane of the EEC cell raise intracellular levels of cAMP and Ca^{2+}, thereby stimulating hormone release from the basolateral membrane [138].

In addition to such nutrient-stimulated hormone release, there is also neural control mediated by the ANS and the enteric nervous system (ENS) [139] (Fig. 3). EEC hormone release is under the control of vagovagal reflexes, and ACh released from VN efferents acts not only at EEC muscarinic ACh receptors (mACh R), but also at the α7nAChR. For

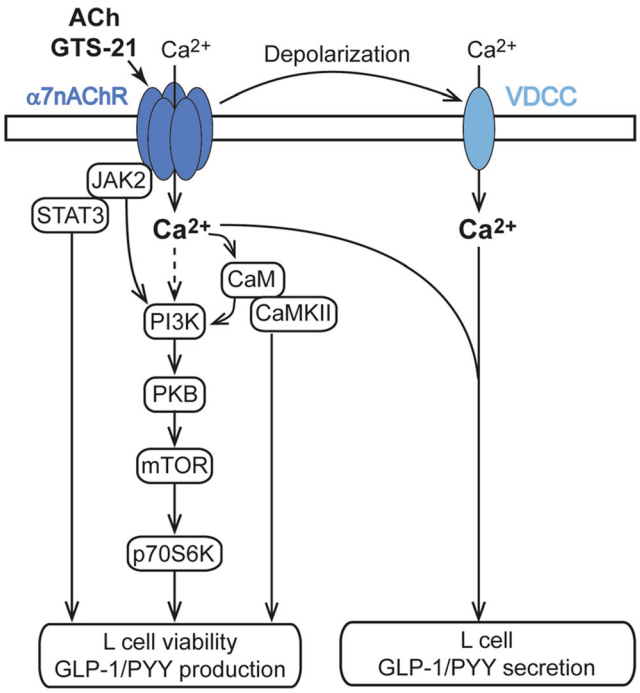

Fig. 3 Dual role of α7nAChR to promote enteroendocrine L-cell GLP-1 and PYY secretion, and to also enhance L-cell viability under conditions of hyperglycemic stress. For additional details see Wang et al. [11]

example, L-cells of the distal intestine synthesize GLP-1 and Peptide YY (PYY) [140], and α7nAChR stimulation results in the co-secretion of both peptides (Fig. 3). Whereas attention previously focused on nutrient control of GLP-1 and PYY release [135], L-cells are innervated by VN efferents [139]. Thus, neural regulation of EEC hormone secretion is of major importance to glucoregulation and appetite control [141], and in fact VN stimulation releases GLP-1 from L-cells [142, 143].

As reported by Wang et al. (2018), studies using RT-PCR, Western blot, or immunofluorescence analysis reveal that α7nAChR is detectable in murine intestine, in the mouse L-cell line GLUTag, the mouse EEC line STC-1, and in the human EEC line NCI-H716 [11]. Double immunofluorescence labeling also demonstrates co-expression of α7nAChR and GLP-1 in primary mouse intestinal cell cultures enriched with L-cells, and in sections of mouse ileum [11]. Moreover, the α7nAChR agonist GTS-21 stimulates GLP-1 release from primary cultures of mouse intestinal cells that are enriched with L-cells [11]. Studies of GLUTag cells demonstrate that this action of GTS-21 results from its ability to promote membrane depolarization, Ca^{2+} influx, and Ca^{2+}-dependent exocytosis of GLP-1 [11] (Fig. 3). As expected, the GLP-1 secretagogue action of GTS-21 is abrogated by pre-treatment with the α7nAChR antagonist α-bungarotoxin, or after siRNA-mediated knockdown of α7nAChR expression [11].

The above-summarized *in vitro* secretagogue actions of GTS-21 correlate with its *in vivo* ability to raise levels of circulating GLP-1 in C57BL/6 mice [11]. More recently, we find that GTS-21 improves oral glucose tolerance in wild-type mice but not α7nAChR KO mice (Q. Meng and R.N. Cooney, unpublished findings). Since GLP-1 is an established blood glucose-lowering hormone [52], this glycemic action of GTS-21 might be indirect (i.e., mediated by GLP-1 released from L-cells). However, an action of GTS-21 independent of L-cell GLP-1 release has not been ruled out. This possibility could be tested in studies using the specific GLP-1 receptor antagonist Ex (9–39) [52]. Defining the exact locus of GTS-21 action relevant to glucoregulation will require a more refined mouse genetics approach using cell type-specific knockouts of α7nAChR in L-cells or other cells types (e.g., ANS or CNS neurons) that express α7nAChR and that might participate in glucoregulation.

GTS-21 also stimulates growth factor signaling pathways in L-cells (Fig. 3). Western blot analysis demonstrates that GTS-21 promotes PI3K, PKB, and mTOR/p70S6K mediated phosphorylation [11]. These effects correlate with the action of GTS-21 to counteract glucotoxicity in an *in vitro* assay of L-cell viability using GLUTag cells [11]. GTS-21 slows apoptosis under conditions of hyperglycemia, and all such actions of GTS-21 are blocked by inhibitors for each of these kinases [11]. Remarkably, buffering of intracellular Ca^{2+} to low levels blocks PI3K, PKB, and mTOR/p70S6K mediated phosphorylation in response to GTS-21 [11]. Conversely, inhibitors of PI3K, PKB, and mTOR/p70S6K suppress Ca^{2+}-dependent GLP-1 secretion in response to GTS-21 [11]. Thus, Ca^{2+} entry through α7nAChR serves as an ionic coupling factor in support of protein kinase-mediate pro-survival and secretagogue actions GTS-21 (Fig. 3). Furthermore, GLP-1 release is conditional on a novel permissive action of PI3K, PKB, and mTOR/p70S6K to support exocytosis.

Interestingly, attempts to ameliorate T2D in clinical studies have not yet been fruitful when testing GLP-1 secretagogues that are GPCR agonists, and that stimulate free fatty acid receptors (TAK-875), or bile acid receptors (SB-756050), or fatty acid amide receptors (APD597) located on L-cells. Potentially, a new therapeutic strategy that is instead based on the use of α7nAChR agonists to restore L-cell number and function will provide a means with which to treat T2D. In fact, the intestinal distribution and numbers of EEC are altered in T2D [144]. Thus, strategies to increase L-cell turnover, number, and differentiated state might be useful as means to treat T2D [144]. In this regard, α7nAChR agonists might be applicable to this purpose, although this has yet to be tested.

The GLP-1 secretagogue action of GTS-21 summarized above is accompanied by its ability to stimulate the release of PYY (1–36) from L-cells [11]. This is significant since circulating PYY (1–36) is converted to PYY (3–36) that transits across the blood brain barrier whereupon it binds to the type 2 isoform of neuropeptide Y receptor (NPY2R) located in the hypothalamus [140]. In this manner, PYY (3–36) exerts an anorexigenic appetite suppressing effect mediated by NPY2R [140]. Simultaneously, GLP-1 excites VN afferents that innervate the NTS, thereby exerting an additional anorexigenic effect [145, 146]. Based on these findings, α7nAChR agonists that simultaneously stimulate GLP-1 and PYY release might find a usefulness for treatment of the metabolic syndrome. If so, they would be predicted to suppress appetite, reduce body weight, enhance liver, fat, and skeletal muscle insulin sensitivity, and improved glycemia in T2D.

There is also a potential role for α7nAChR as a determinant of GLP-1 release from α-cells located in the islets of Langerhans. Normally, these α-cells release glucagon, a hormone that stimulates hepatic glucose production to counteract hypoglycemia. However, under conditions of stress resembling T2D, a phenotypic conversion occurs in which α-cells attain the ability to synthesize and release GLP-1 [147–149]. This intra-islet GLP-1 then acts as a paracrine hormone to stimulate GLP-1 receptors located on β-cells within the islets [147]. Resultant GLP-1 receptor activation not only protects β-cells from apoptosis induced by glucolipotoxicity [150, 151], but it also enhances insulin biosynthesis and secretion [152–155]. Recently, we established an ability of GTS-21 to stimulate GLP-1 release from the mouse α-cell line αTC1.6.

Furthermore, we find that GTS-21 upregulates prohormone convertase (PC1/3) expression to stimulate conversion of proglucagon to GLP-1 (O.G. Chepurny and Q. Meng, unpublished findings). Thus, α7nAChR agonists might serve as intra-islet GLP-1 secretagogues for treatment of T2D.

8 α7nAChR regulation of β-cell adaptation to stress

Preganglionic VN efferents in rats and mice innervate pancreatic ganglia where they synapse on postganglionic neurons that project to the islets of Langerhans [156]. ACh released from these VN efferents stimulates insulin secretion from islet β-cells [157]. Furthermore, this ACh exerts a proliferative effect to stimulate β-cell replication in rats and mice so that β-cell "mass" is increased [158]. In humans there is VN control of insulin secretion [159, 160], but cholinergic VN innervation of human islets is sparse [161]. Instead, ACh is released from human islet α-cells so that it acts as a paracrine transmitter to stimulate ACh receptors on β-cells [162]. ACh action at β-cells is mediated by assemblies of nAChR isoforms comprised of α2, α3, α4, α5, α7, β2, and β4 subunits, of which α7 and β2 are most abundantly expressed in mouse islets [10, 163–165]. ACh also stimulates multiple isoforms of β-cell mAChR, of which the M3 mAChR is of high significance [166] (Fig. 4).

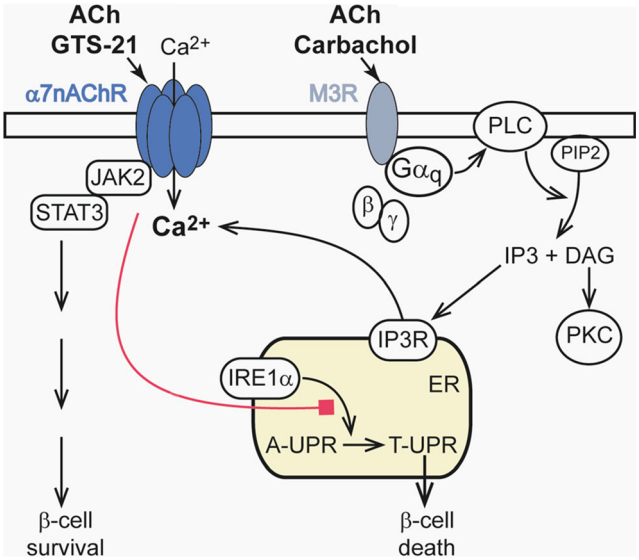

Fig. 4 The nicotinic α7nAChR mediates actions of ACh to counteract endoplasmic reticulum stress and to protect against apoptosis in pancreatic β-cells. However, Ca^{2+} entry through α7nAChR fails to stimulate β-cell insulin secretion. Instead, ACh action at muscarinic M_3 receptors promotes insulin secretion through the mobilization of intracellular Ca^{2+}, while also upregulating protein kinase C (PKC) activity. Abbreviations: A-UPR, adaptive unfolded protein response; T-UPR, terminal unfolded protein response; IRE1α, inositol-requiring enzyme 1α

Paradoxically, nicotine inhibits insulin release rather than stimulating it [163, 165]. This indicates that nAChR activation fails to sufficiently raise levels of [Ca^{2+}] within "active zones" of exocytosis where insulin secretion occurs [167]. However, "priming" of islets with nicotine enhances the ability of mAChR agonist oxotremorine to stimulate insulin secretion [166]. Such findings indicate that nAChR function in β-cells is not conventional with respect to standard secretagogue actions of nicotine and ACh observed in other endocrine cell types (e.g., adrenal chromaffin cells that secrete epinephrine). Thus, the question arises as to what role α7nAChR plays in β-cell biology? As summarized below, the β-cell α7nAChR mediates a trophic factor-like action of α7nAChR agonists to counteract apoptosis and to enhance β-cell survival in mouse models of diabetes (Fig. 4).

Gupta et al. provide the most definitive study validating an ability of an α7nAChR agonist (PNU 282987) to counteract hyperglycemia, suppress β-cell apoptosis, and to preserve β-cell mass (BCM) through a β-cell pro-survival effect that is monitored in the multiple low-dose streptozotocin (MLDS) model of diabetes using mice [10]. In this model, steptozotocin (STZ) induces β-cell apoptosis and reduces BCM, while also inducing an inflammatory response (insulitis) in which immune cells release cytokines that further accelerate β-cell death [168]. This MLDS model resembles, but does not fully recapitulate, some features of autoimmune type 1 diabetes (T1D) in which immune destruction of β-cells leads to insulin insufficiency and sustained hyperglycemia [168].

When PNU 282987 is administered for 2 weeks on a daily basis to MLDS mice, it exerts a significant long-term blood glucose-lowering effect measured in an intra-peritoneal glucose tolerance test [10]. This action of PNU 282987 is attributable to its ability to protect against the decrease of BCM that streptozotocin induces. Thus, PNU 282987 partially reverses insulin insufficiency in the MLDS model [10]. TUNEL assays with MLDS pancreas slices reveal that PNU 282987 preserves BCM by counteracting STZ-induced apoptosis [10]. Mechanistically, PNU 282987 stimulates the JAK2-STAT3 anti-inflammatory signaling pathway, and it also upregulates PKB, BCL2, and CREB pro-survival signaling pathways [10]. Importantly, these effects are measured in islets of wild-type mice but not α7nAChR KO mice [10]. PNU 282987 also counteracts streptozotocin-induced depletion of β-cell transcription factors Pdx1 and MafA that are important to establishment of β-cell identity in the fully differentiated state [10] (refer to Fig. 2). Thus, PNU 282987 preserves gene expression important to β-cell survival, while also preserving the ability of β-cells to synthesize and release insulin [10].

Additional β-cell anti-apoptosis actions of PNU 282987 and nicotine are reported by Ishibashi et al. for studies of the rat β-cell line INS-1 and the human β-cell line EndoC-βH1 [169]. These studies focus on how α7nAChR stimulation

counteracts the "terminal" unfolded protein response (T-UPR) that induces apoptosis, and that results from excessive endoplasmic reticulum (ER) stress [170] (Fig. 4). In healthy β-cells there normally exists an "adaptive" UPR (A-UPR) that does not induce apoptosis, and that is instead cytoprotective in that it monitors secretory protein folding and assembly in the ER. When secretory protein demand increases (e.g., upregulation of ER proinsulin content), the A-UPR is activated to enhance ER protein folding capacity, to degrade ER unfolded proteins, and to reduce the "load" of secretory proteins transported to the plasma membrane for exocytosis [170]. However, under conditions of excessive β-cell ER stress (e.g., failure of ER proinsulin to fold properly), the T-UPR is initiated with consequent β-cell apoptosis [170]. A key event underlying the T-UPR is hyperactivation of the UPR sensor IRE1α (inositol-requiring enzyme 1α) [170]. This leads to hyperactivation of endoribonuclease (RNase), cleavage of ER-localized mRNAs, ribosomal RNAs, and micro RNAs, so that apoptosis is initiated [170]. Ishibashi et al. find that nicotine inhibits IRE1α activation in response to ER stress [169]. Specifically, induction of T-UPR by treatment with tunicamycin (an inhibitor of ER protein folding) leads to an autophosphorylation of IRE1α that signals its activation, an effect inhibited by treatment with nicotine [169]. This effect of nicotine correlates with its ability to suppress tunicamycin-induced upregulation of the T-UPR markers XBP1s (spliced X-box binding protein) and TXNIP (thioredoxin-interacting protein). PNU 282987 mimics these cytoprotective actions of nicotine, and these effects are absent after siRNA knockdown of α7nAChR [169].

As summarized above, α7 and β2 are the predominant isoforms of nAChR subunits found in mouse islets. Thus, it is significant that Somm et al. report that glucose-stimulated insulin secretion is not impaired in islets of α7β2nAChR mice in which there is a double KO of α7 and β2 [164]. This finding reinforces the view that α7β2nAChR action in mouse islets is unrelated to short-term control of insulin secretion. Importantly, Klee et al. further substantiate the long-term trophic role α7 plays by demonstrating an ability of nicotine to exert a pro-survival effect to counteract cytokine-induced apoptosis in islets of mice with a KO of β2 but not α7 [171]. These investigators also report a major species difference in which α5 mRNA is expressed at higher levels than α7 in human islets [171]. However, this analysis is open to interpretation since whole islets contain multiple endocrine cell types. Ideally, such studies will be repeated using pure preparations of β-cells [171].

Studies of Klee et al. also shed light on the physiological basis of α7nAChR agonist action in β-cells. In mouse islets, α7nAChR agonist choline reduces cytokine-induced activation of caspase3, a mediator of apoptosis [171]. Furthermore, choline counteracts cytokine-induced elevation of $[Ca^{2+}]_i$, endoplasmic reticulum stress, and mitochondrial outer membrane permeabilization (MOMP), all of which are key events leading to apoptosis. Collectively, these findings are of potential medical significance relevant to T1D since choline also counteracts apoptosis induced by treatment of human islets with pro-inflammatory cytokines (IL-1β, TNFα, IFNγ) [171]. Still, the direct involvement of β-cell α7nAChR as the principal molecular target of choline action in human β-cells remains not fully established in the absence of a targeted KO or knockdown of the various α subunits expressed within human β-cells.

9 Emerging clinical strategies relevant to anti-obesity therapeutics

An increasingly popular clinical procedure for treatment of obesity is based on the use of implantable electrical devices that allow chronic stimulation of the vagus nerve [21]. These implantable VNS devices are safe and effective so that a 12-month stimulation regimen provides excess weight loss of ca. 25%, an effect accompanied by rapid improvements in glycemic control and blood pressure that are long lasting [172]. The efficacy of VNS for weight reduction is understandable in view of the above summarized roles of CAIR, vagovagal reflexes, and α7nAChR to control metabolic homeostasis. However, disadvantages of VNS include the need for implant surgery and the high cost of the implantable device. A small percentage of patients complain of pain at the neuroregulatory site, and mild to moderate symptoms of heartburn, dysphagia and/or nausea [48]. Replacement of device batteries and electrodes is also a complicating factor. Still, this approach is substantially less intrusive than bariatric surgery options such as gastric bypass surgery or sleeve gastrectomy for treatment of obesity. What is presently unknown is whether the efficacy of VNS for weight reduction might be enhanced by co-administration of an α7nAChR agonist. This combinatorial approach is plausible in view of prior clinical testing of investigational α7nAChR agonists that were demonstrated to be generally well tolerated. For example, there was suitable patient compliance when evaluating α7nAChR agonist efficacy for treatment of schizophrenia and cognitive dysfunction [59]. Such studies established that GTS-21 is generally well tolerated, with a low frequency of adverse side effects (eczema, dermatitis, dizziness, headache, and orthostatic hypotension) when it is administered at doses of up to 450 mg/day [173].

Finally, it is of note that an alternative approach targeting CNS cholinergic neurotransmission is based on the use of the AChE inhibitor galantamine. It slows hydrolytic degradation of ACh so that it exerts a generalized effect to stimulate multiple AChR subtypes including muscarinic and nicotinic ACh receptors [119]. In a 12-week clinical trial, galantamine attenuates inflammation, reduces insulin resistance, and improves glycemia [119]. Galantamine is FDA-approved to treat

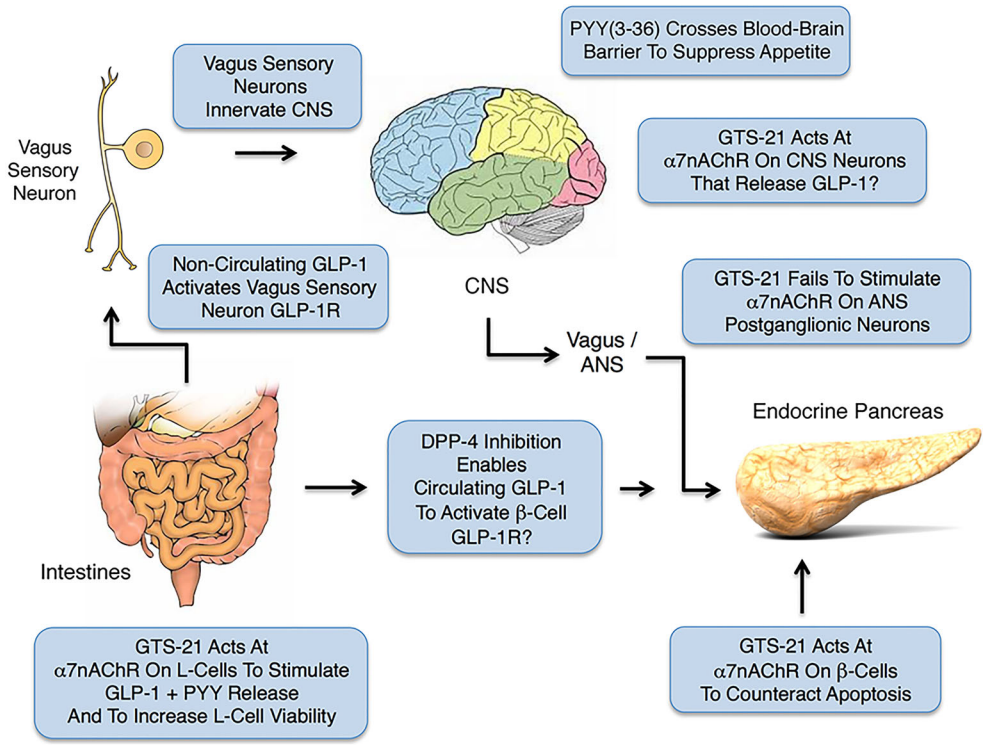

Fig. 5 α7nAChR agonist GTS-21 participates in the control of GLP-1 and PYY release from L-cells, while also protecting β-cells from apoptosis. Note that GLP-1 released from L-cells initiates vagovagal reflexes that transmit through CNS and ANS to influence whole-body metabolism, whereas PYY crosses the blood brain barrier to suppress appetite. Dipeptidylpeptidase-4 (DPP-4) inhibitors enhance the glycemic action of GLP-1 by slowing its normally fast metabolic inactivation. It is presently uncertain whether levels of circulating GLP-1 are sufficiently high to stimulate the β-cell GLP-1 receptor even when T2D patients are administered DPP-4 inhibitors. Instead, DPP-4 inhibitors may primarily enhance GLP-1 action at the GLP-1R located on VN afferents. For additional details see Burcelin et al. [146]

dementia, and it is well tolerated, with only three adverse events in six study patients [119]. However, nausea, vomiting, diarrhea, dizziness, and weight loss are infrequently reported. Intriguingly, anti-inflammatory effects of galantamine appear to be mediated by its CNS site of action at ACh receptors in which it upregulates efferent VN outflow that emulates CAIR [17]. Therefore, it will be interesting to evaluate to what extent α7nAChR participates in the beneficial metabolic actions of galantamine reported to date.

10 Conclusion

A principal conclusion to be drawn from this review of the literature is that vagovagal reflexes are mediated by shared assemblies of VN afferent and efferent circuits that are of importance to homeostatic control of inflammation, appetite, body weight, energy expenditure, and glycemia (Fig. 5). VN sensory input that contributes to this control is relayed through the CNS and ANS, and it is initiated by direct actions of inflammatory cytokines, nutrients, metabolites, and hormones binding to their receptors located on the peripheral terminations of VN afferents in multiple organ systems. α7nAChR plays an important role as a determinant of VN efferent control of immune cell function, while it also participates in the ANS regulation of EEC hormone secretion (GLP-1, PYY), intestinal L-cell viability, and pancreatic β-cell survival. Here, we propose that defects of the vagal reflex circuitry underlying CAIR explain, at least in part, why inflammation, obesity, and diabetes coexist in the metabolic syndrome. Potentially, such defects may be overridden by direct electrical stimulation of the VN in order to achieve a therapeutic effect. However, a pharmacological approach using α7nAChR agonists might achieve a similar outcome. To date, α7nAChR is primarily considered to be a target for drug discovery relevant to treatment of neurodegenerative and psychiatric disorders. Still, absent their potential deleterious side effects owing to CNS, ANS, or ENS α7nAChR activation, available evidence indicates a likely role for investigational α7nAChR agonists as a new experimental treatment for the metabolic syndrome.

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