An α7 Nicotinic Acetylcholine Receptor-Selective Agonist Reduces Weight Gain and Metabolic Changes in a Mouse Model of Diabetes

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ABSTRACT

Type 2 diabetes has become a pervasive public health problem. The etiology of the disease has not been fully defined but appears to involve abnormalities in peripheral and central nervous system pathways, as well as prominent inflammatory components. Because nicotinic acetylcholine receptors (nAChRs) are known to interact with anti-inflammatory pathways and have been implicated in control of appetite and body weight, as well as lipid and energy metabolism, we examined their role in modulating biological parameters associated with the disease. In a model of type 2 diabetes, the homozygous leptin-resistant db/db obese mouse, we measured the effects of a novel α7 nAChR-selective agonist [5-methyl-N-[2-(pyridin-3-ylmethyl)-1-azabicyclo[2.2.2]oct-3-yl]thiophene-2-carboxamide (TC-7020)] on body mass, glucose and lipid metabolism, and proinflammatory cytokines. Oral administration of TC-7020 reduced weight gain and food intake, reduced elevated glucose and glycated hemoglobin levels, and lowered elevated plasma levels of triglycerides and the proinflammatory cytokine tumor necrosis factor-α. These changes were reversed by the α7-selective antagonist methyllycaconitine, confirming the involvement of α7 nAChRs. Prevention of weight gain, decreased food intake, and normalization of glucose levels were also blocked by the Janus kinase 2 (JAK2) inhibitor α-cyano-(3,4-dihydroxy)-N-benzylcinnamide (AG-490), suggesting that these effects involve linkage of α7 nAChRs to the JAK2-signal transducer and activator of transcription 3 signaling pathway. The results show that α7 nAChRs play a central role in regulating biological parameters associated with diabetes and support the potential of targeting these receptors as a new therapeutic strategy for treatment.

In 2000 it was reported that at least 171 million people worldwide (2.8% of the population) suffered from diabetes, and it has been estimated that the incidence will almost double by the year 2030 (Wild et al., 2004). The Centers for Disease Control and Prevention has designated the disease an epidemic. It has been estimated that the incidence will almost double by the year 2030 (Wild et al., 2004). The Centers for Disease Control and Prevention has designated the disease an epidemic. In general, diabetes is believed to be secondary to an insulin-resistant state, which is associated with excess adiposity (Sykiotis and Papavassiliou, 2001). Insulin resistance in skeletal muscle, liver, and adipose tissue impedes glucose uptake and results in the release of free fatty acids and the characteristically associated dyslipidemia. Elevations in postprandial blood glucose levels and ultimately in fasting glucose levels result in compensatory hyperinsulinemia, a condition that is initially accompanied by islet β-cell hypertrophy and eventual failure (Sykiotis and Papavassiliou, 2001).

A key factor that underlies the development of diabetes is a characteristic systemic inflammation, marked by increases in the venous blood concentrations of C-reactive protein, interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α) (Bulló et al., 2003). TNF-α has been shown not only to evoke the production of other inflammatory cytokines but also to increase the activities of signaling pathways that are believed to lead to insulin resistance (Dandona et al., 2004). The central nervous system (CNS) modulates inflammation, including levels of TNF-α, via...

ABBREVIATIONS: IL-6, interleukin 6; TNF-α, tumor necrosis factor-α; CNS, central nervous system; ACh, acetylcholine; nAChR, nicotinic acetylcholine receptor; LPS, lipopolysaccharide; TC-7020, 5-methyl-N-[2-(pyridin-3-ylmethyl)-1-azabicyclo[2.2.2]oct-3-yl]thiophene-2-carboxamide; Compound A, (R,E)-5-(2-pyrrolidin-3-ylvinyl)pyrimidine; MLA, methyllycaconitine; JAK2, Janus kinase 2; AG-490, α-cyano-(3,4-dihydroxy)-N-benzylcinnamide; HbA1c, glycylated hemoglobin; STAT3, signal transducer and activator of transcription 3.
the reticuloendothelial system. The vagus nerve, using its major neurotransmitter acetylcholine (ACh), acts on α7 nicotinic acetylcholine receptors (nAChRs) of macrophages to suppress TNF-α release (Borovikova et al., 2000a; Miao et al., 2003; Wang et al., 2003). Electrical stimulation of the vagus nerve or treatment of vagotomized animals with ACh prevents lipopolysaccharide (LPS)-dependent increases in TNF-α release (Borovikova et al., 2000b). Conversely, vagotomy increases TNF-α serum levels and hepatic TNF-α responses (Borovikova et al., 2000b). The role of α7 nAChRs in cholinergic modulation of TNF-α in macrophages has been confirmed using antisense oligonucleotides to the α7 nAChR (Wang et al., 2003). Indeed, when the expression of this receptor is prevented, ACh loses its effect on LPS-induced TNF-α release. Furthermore, stimulation of the vagus nerve does not inhibit TNF-α release in α7 knock-out mice (Wang et al., 2003). The key role played by α7 nAChRs in inflammatory processes is further supported by the observations that nicotine and α7 nAChR agonists are effective in models of inflammation and protective in models of sepsis and that they inhibit local leukocyte recruitment and decrease endothelial cell activation (de Jonge and Ulloa, 2007).

Obesity is also a major predisposing factor in the development of diabetes. Relevant to this is an extensive literature on the nonselective nAChR agonist nicotine supporting a broad involvement of both CNS and peripheral nAChRs in regulating body mass and other key metabolic pathways. It is well known that nicotine administration decreases body weight in normal rodents and human smokers and results in adaptive changes that regulate feeding behavior and energy metabolism (Fornari et al., 2007). Nicotine has also been shown to reduce the incidence of type I diabetes in mice (Mabley et al., 2002) and improve insulin sensitivity in rat adipocytes (Liu et al., 2004). Nicotine influences expression of the orexigenic peptides neuropeptide Y and Agouti-related protein in the hypothalamus, as well as the expression of the metabolic protein, uncoupling protein-3 in brown adipose tissue (Fornari et al., 2007). Areas of the hypothalamus, particularly the lateral hypothalamus that regulates appetite, contain α7 nAChRs, which have been postulated to play a key role in regulating appetite, food consumption, and body mass (Jo et al., 2002).

To more precisely probe the relationship of the α7 nAChR to specific physiological components of diabetes we have designed and synthesized a novel agonist (TC-7020) with high selectivity for the α7 nAChR. The effects of this compound were studied in an animal model of type 2 diabetes, the db/db mouse, which expresses many of the pathologic changes associated with the disease, including hyperglycemia, hyperlipidemia, increased body weight, increased TNF-α levels in adipose tissue, and nephropathy (Hotamisligil et al., 1993; Harris et al., 2001; Sharma et al., 2003). The results indicate that activation of α7 nAChR targets by this compound significantly reverses weight gain and associated metabolic changes expressed in the leptin receptor-deficient db/db mouse.

### Materials and Methods

**α7 and α4β2 nAChR-Selective Compounds**

TC-7020 (α7). TC-7020 was prepared from commercially available quinuclidin-3-one by aldol condensation with 3-pyridinecarboxaldehyde to afford 2-[(pyridin-3-yl)methylene]quinuclidin-3-one, followed by catalytic hydrogenation. The carbonyl moiety of the resulting 2-[(pyridin-3-yl)methyl]quinuclidin-3-one was converted into amino group by reductive amination. Final coupling of 3-amino-2-[(pyridin-3-yl)methyl]-1-azabicyclo[2.2.2]octane with 5-methylthiophen-2-carboxylic acid provided TC-7020 (see Fig. 1 for structure).

**Compound A (α4β2).** Compound A is (R,E)-5-(2-pyrrolidin-3-ylation)-pyrimidine.

### Receptor Binding Assays

[^3H]Nicotine binding to α4β2 nAChRs in rat cortical membrane preparations was assayed using standard methods adapted from published procedures (Lippiello and Fernandes, 1986).[^3H]Methyllycaconitine (MLA) binding to α7 nAChRs was determined in hippocampal membranes as described previously (Davies et al., 1999). The IC₅₀ (concentration of the compound that produces 50% inhibition of binding) was determined by least-squares nonlinear regression using GraphPad Prism software (GraphPad Software Inc., San Diego, CA). Kᵢ was calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

### Patch-Clamp Electrophysiology

**Expression in Xenopus Oocytes.** Mature (>9 cm) female Xenopus laevis African toads (Nasco, Fort Atkinson, WI) were used as a source of oocytes. After linearization and purification of cloned cDNAs, RNA transcripts were prepared in vitro using the appropriate mMESSAGE mMACHINE kit from Ambion (Austin, TX). Stage 5 oocytes were isolated and injected with 50 nl (5–20 ng) each of the appropriate subunit cRNAs. Recordings were made 2 to 7 days after injection.

**Electrophysiology.** Patch-clamp electrophysiology studies of the human α7 nAChR using X. laevis oocytes were performed in the laboratory of Roger Papke (University of Florida, Gainesville, FL). Experiments were conducted using the OpusXpress 6000A (Axon Instruments, Sunnyvale, CA). Responses were calculated using a net charge analysis for α7 receptors (Papke and Porter Papke, 2002). For concentration-response relations, data derived from net charge analyses were plotted using Kaleidagraph 3.0.2 (Abelbeck/Synergy, Reading, PA), and curves were generated from the Hill equation:

\[
\text{Response} = \frac{I_{\text{max}} \times [\text{agonist}]^{I_{\text{H}}}}{IC_{50}^{I_{\text{H}}} + [\text{agonist}]^{I_{\text{H}}}}
\]

where \(I_{\text{max}}\) denotes the maximal response for a particular agonist/subunit combination, and \(n\) represents the Hill coefficient. \(I_{\text{max}}\), \(n\), and the \(IC_{50}\) were all unconstrained for the fitting procedures.

### Receptor Function Assays

TE671/154 and SH-SY5Y cell lines (obtained from Dr. Ron Lukas, Barrow Neurological Institute, Phoenix, AZ) and the SH-EP1 cell line were used to assess the functional properties of the muscle, ganglionic, and α4β2 nAChR subtypes, respectively. Cells were maintained in proliferative growth phase in Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA) with 10% horse serum (Invitrogen), 5% fetal bovine serum (HyClone Laboratories, Logan UT), 1 mM sodium pyruvate, and 4 mM l-glutamine. Forty-eight
hours before each experiment, cells were plated in 96-well black-walled plates (Corning Inc., Corning, NY) at 60,000 cells/well. On the day of the experiment, growth medium was gently removed; 200 μL of FLIPR Calcium 4 Assay reagent (Molecular Devices, Sunnyvale, CA) in assay buffer (10 mM HEPES, 2.5 mM CaCl₂, 5.6 mM d-glucose, 0.8 mM MgSO₄, 5.3 mM KCl, 138 mM NaCl, pH 7.4, with Tris base) was added to each well; and plates were incubated at 37°C for 1 h. The plates were removed from the incubator and allowed to equilibrate to room temperature for at least 30 min. Plates were transferred to a Flexstation fluorescence plate reader (Molecular Devices) for addition of compound and monitoring of fluorescence at 485 nm. The amount of calcium flux was compared with both a positive (10 μM nicotine) and negative control (buffer alone) to determine the percentage response relative to that of nicotine. The positive control was defined as 100% response, and the results of the test compounds were expressed as a percentage of the positive control.

Animals

Mice used in these studies included C57BL/6J heterozygous lean controls (referred to herein as db) and leptin receptor-deficient (referred to herein as db⁻) mice on a C57BL/6J background; both were obtained from The Jackson Laboratory (Bar Harbor, ME). Animals had ad libitum access to drinking water and rodent chow. Studies were conducted in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) as adopted and promulgated by the National Institutes of Health.

Drug Treatment

The effects of the α7-selective agonist (TC-7020) on body weight and food intake were measured biweekly from ages 3 to 10 weeks. TC-7020 was given via gavage at 1 mg/kg daily. The Janus kinase 2 (JAK2) kinase inhibitor (AG-490) and food intake were measured biweekly from ages 3 to 10 weeks.

Table 1: In vitro pharmacological profile of TC-7020 and α4β2-selective compound A

<table>
<thead>
<tr>
<th>nAChR Subtype</th>
<th>Source</th>
<th>Parameter</th>
<th>TC-7020</th>
<th>Compound A</th>
</tr>
</thead>
<tbody>
<tr>
<td>α7</td>
<td>Rat hippocampus (Oocytes (voltage clamp))</td>
<td>Kᵢ (nM)</td>
<td>2.0 ± 0.3</td>
<td>5500 ± 1900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EC₅₀ (% ACh)</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>α4β2</td>
<td>Rat cortex (Human (SH-EP1 cells))</td>
<td>Kᵢ (nM)</td>
<td>69 ± 4*</td>
<td>4166 ± 1851</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EC₅₀ (nM) Ca²⁺ flux</td>
<td>N.D.</td>
<td>27 ± 11</td>
</tr>
<tr>
<td>Muscle</td>
<td>Human (SH-SY5Y cells)</td>
<td>EC₅₀ (nM) Ca²⁺ flux</td>
<td>1.3 ± 0.8</td>
<td>120 ± 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eₘₐₓ (%nicotine)</td>
<td>6.5 ± 4.0</td>
<td>12 ± 4*</td>
</tr>
<tr>
<td>Ganglion</td>
<td>Human (TE-671 cells)</td>
<td>Ca²⁺ flux (%nicotine at 100 μM)</td>
<td>5.8 ± 1.2</td>
<td>32 ± 12*</td>
</tr>
</tbody>
</table>

N.D., Not determined.

* Value determined for racemate.

* The values are consistent with the percentage inhibition at 10 μM for the nicotinic, neuronal (α₂β₄δαoinsensitive) receptor (Supplemental Table 1).

Results

In Vitro Pharmacology

TC-7020 is a novel proprietary agonist that is highly selective for the α7 nAChR subtype, based on both binding affinity and function (Table 1). The compound binds to α7 nAChRs with high affinity (Kᵢ ~2 nM in displacement studies using [³H]MLA in rat hippocampal synaptosomes) and exhibits very poor affinity toward other nicotinic receptor subtypes (Kᵢ >1000 nM), including the other major subtype in brain (α4β2). In functional studies, TC-7020 is an agonist at α7 nAChRs (Eₘₐₓ 69%), as evidenced by voltage-clamp studies of human α7 nAChRs transiently expressed in Xenopus oocytes. TC-7020 showed minimal functional activity at other muscle (~7% of nicotine’s Eₘₐₓ at 100 μM) or ganglionic (~6% of nicotine’s Eₘₐₓ at 100 μM) nAChR receptor subtypes, as shown by measuring calcium flux in SH-SY5Y cells and TE-671 cells, respectively. TC-7020 does not exhibit selectivity for any other (non-nicotinic) receptor targets (IC₅₀ >10 μM at more than 60 targets in a broad receptor selectivity panel; Supplemental Table 1). Although there was a slight binding to human ether-a-go-go-related gene channels (21% at 10 μM), a follow-up functional assay showed that the EC₅₀ was >100 μM.

Physiological Effects of Selective α7 nAChR Agonist

Plasma TNF-α Levels. It has been shown that the plasma concentrations of inflammatory mediators such as TNF-α are increased in the insulin-resistant diabetic state, and that the reduction of the levels of TNF-α in diabetic mice correlates with increased insulin sensitivity and decreased plasma insulin and blood glucose levels (Uysal et al., 1997). Therefore, we determined the effects of the α7 agonist on obesity-induced levels of plasma TNF-α. TC-7020-treated and untreated lean db⁻ mice showed no change in the plasma levels of TNF-α, but obese db⁻ mice had elevated fasting plasma TNF-α levels. When the db⁻ obese mice were treated with TC-7020, they displayed significantly decreased...

Data Analyses

All the data are expressed as mean ± S.E.M. Differences among all the groups were compared using a two-way analysis of variance. Where significant main effects were shown, post hoc analyses with Tukey’s multiple comparisons test were performed to determine significant differences between treatment groups. For all the analyses, an α level of 0.05 was considered statistically significant.
plasma TNF-α levels compared with their vehicle-treated controls ($p < 0.05$). However, levels did not return to those of lean controls. The decrease was blocked by the α7 antagonist MLA (Fig. 1), implicating the involvement of α7 nAChRs.

**Glucose Metabolism.** Because weight is known to correlate with glucose metabolism and insulin sensitivity in obesity and diabetes (Williams et al., 2003), we assessed plasma glucose levels in the treated and untreated db⁻/⁻ obese mice. Lean TC-7020-treated and untreated db⁻/⁻ mice all showed normal glucose levels. At the end of 7 weeks of treatment, fasting plasma glucose levels in the db⁻/⁻ obese mice treated with the α7 agonist were significantly lower ($p < 0.05$) than those in the vehicle-treated db⁻/⁻ mice (Fig. 2A). Levels did not return to those of lean controls. When the α7 nAChR antagonist MLA was given concurrently with TC-7020, the obese mice showed no significant decrease in plasma glucose, indicating that the effects on glucose levels are dependent either directly or indirectly on α7 nAChR activation. The effects on plasma glucose level also appear to be dependent on JAK2 activation, as shown by the finding that the JAK2 inhibitor AG-490 prevented the TC-7020-induced decrease in plasma glucose (Fig. 2B).

Because total glycemic load includes both fasting and postprandial glucose levels in the blood, a time-averaged index of glycemic load is reflected in the accumulation of advanced glycation end products, as exemplified by the quantitative glycosylation of hemoglobin, HbA1c. Lean TC-7020-treated and untreated db⁻/⁻ mice showed normal glycemic control. In contrast, obese db⁻/⁻ mice showed markedly elevated HbA1c levels, and these levels were significantly lowered ($p < 0.05$) by TC-7020. These observations indicate that the α7 nAChR plays a central role in regulating both the fasting and postprandial glucose levels in the blood. With this in mind, coadministration of the α7 antagonist MLA suppressed the reduction in HbA1c levels induced by the α7 agonist TC-7020 (Fig. 3).

**Lipid Metabolism.** The nonselective nAChR agonist nicotine has been shown to have effects on peripheral (non-neural) sites of energy metabolism, including decreased lipolysis and decreased triglyceride uptake and storage in adipose tissue (Jo et al., 2002). Therefore, to explore the involvement of α7 nAChRs in modulating lipid metabolism we monitored the effects of TC-7020 on plasma triglyceride levels. Lean TC-7020-treated and untreated db⁻/⁻ mice all showed normal levels of triglycerides. Obese db⁻/⁻ mice displayed elevated fasting triglyceride levels, consistent with a loss of insulin sensitivity in adipocytes. When the db⁻/⁻ obese mice were treated with TC-7020, there was a marked reduction of elevated triglyceride levels compared with vehicle-treated obese controls ($p < 0.05$), but levels did not return to those of lean controls. The effects of TC-7020 were blocked by the α7 antagonist MLA (Fig. 4), suggesting modulation of lipid metabolism and possibly of adipocyte insulin resistance via an α7 nAChR-mediated pathway.

**Body Weight Gain and Food Consumption.** The relatively nonselective nAChR agonist nicotine is well known to have effects on body mass, a phenomenon well illustrated by the lower average weight of smokers. These effects of nicotine have been linked to changes in feeding behavior and increased energy metabolism (Fornari et al., 2007), presumably mediated by nAChR subtypes that have been identified in relevant pathways in the CNS and periphery (Jo et al., 2002). Because the db⁻/⁻ mouse expresses an obese phenotype, we probed the role of the α7 nAChR subtype in regulating weight gain by monitoring the effects of the α7-selective agonist
Controls; #, p < 0.05, significantly different from lean vehicle controls; †, p > 0.05, not significantly different from obese vehicle controls.

TC-7020 on body mass. At the end of 7 weeks of treatment, between ages 3 and 10 weeks, weight gain in the vehicle control db⁻ obese groups was significantly greater (p < 0.05) than that of lean vehicle controls (Fig. 5A) as expected. By comparison, weight gain was significantly reduced (p < 0.05) in the α7 agonist-treated db⁻ obese mice (Fig. 5A). The daily food intake in vehicle control obese groups was significantly greater (p < 0.05) than that of lean vehicle controls and was significantly lower (p < 0.05) in the TC-7020-treated obese mice than in the obese controls (Fig. 5B). The food consumption and body mass of the db⁺ lean mice were unaffected by TC-7020 or MLA, confirming that the compounds were not producing toxic effects that altered food intake or weight. However, when the selective α7 antagonist MLA was given concurrently with TC-7020, the obese mice showed no significant differences in body weight gain or food intake compared with the obese vehicle-treated controls (Fig. 6, A and B), confirming that the reduced weight gain is mediated by α7 nAChRs.

Previous studies have shown that α7 nAChRs are linked to antiapoptotic and anti-inflammatory effects through JAK2/ signal transducer and activator of transcription 3 (STAT3) signaling pathways (Marrero and Bencherif, 2009); therefore, to determine whether this pathway is also involved in the observed effects on food consumption and weight loss, we used the JAK2 tyrosine kinase specific inhibitor AG-490. AG-490 prevented (p < 0.05) both the weight loss and the decreased food intake in obese db⁻ mice treated with the α7 agonist TC-7020 (Fig. 7, A and B).

**Specificity of α7 versus α4β2-Selective Ligands**

To further explore the role of nAChR subtypes in modulating parameters of the metabolic syndrome, we compared the effects of a full agonist with high selectivity for the CNS α4β2 nAChR subtype (Compound A) with those of TC-7020. The in vitro profile of Compound A is summarized in Table 1. The results from in vivo studies (Table 2) confirmed the effects of the α7-selective compound TC-7020 on weight gain reduction, reduction of increased glucose levels, decreased glycation of hemoglobin, reduction of the proinflammatory cyto-

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**Fig. 4.** Effects of the α7 nAChR agonist TC-7020 and the α7 nAChR antagonist MLA on glycated hemoglobin levels. Both lean (db⁻) and obese (db⁻) mice were treated for 7 weeks either with vehicle (V) or TC-7020 (TC). In some experiments obese mice were treated with TC-7020 plus the α7 antagonist MLA. TC-7020 (1 mg/kg) and MLA (3 mg/kg) were administered daily via oral gavage. Results represent the mean ± S.E.M. of eight treated mice and are expressed as fasting percentage of HbA1c levels at week 7. *, p < 0.05, significantly different from lean vehicle controls; †, p < 0.05, significantly different from obese vehicle controls.

**Fig. 5.** Effects of the α7 nAChR agonist TC-7020 and the α7 nAChR antagonist MLA on plasma triglycerides. Both lean (db⁻) and obese (db⁻) mice were treated for 7 weeks either with vehicle (V) or TC-7020 (TC). In some experiments, obese mice were treated with TC-7020 plus the α7 antagonist MLA. TC-7020 (1 mg/kg) and MLA (3 mg/kg) were administered daily via oral gavage. Results represent the mean ± S.E.M. of eight treated mice and are expressed as plasma triglyceride levels in milligrams per deciliter. *, p < 0.05, significantly different from lean vehicle controls; †, p > 0.05, not significantly different from obese vehicle controls.

**Fig. 6.** Effects of the α7 nAChR agonist TC-7020 and α7 nAChR antagonist MLA on body mass and food consumption. Both lean (db⁻) and obese (db⁻) mice were treated for 7 weeks either with vehicle (V) or TC-7020 (TC). In some experiments, obese mice were treated with TC-7020 plus the α7 antagonist MLA. TC-7020 (1 mg/kg) and MLA (3 mg/kg) were administered daily via oral gavage. Results represent the mean ± S.E.M. of eight treated mice and are expressed as their body mass in grams (A) or average food consumption in grams/day (B) at week 7. *, p < 0.05, significantly different from lean vehicle controls; †, p < 0.05, significantly different from obese vehicle controls; †, p > 0.05, not significantly different from obese vehicle controls.
weight gain in a murine model of type 2 diabetes. These effects were reversed by the α7 antagonist MLA. Furthermore, the JAK2 kinase-specific inhibitor AG-490 also inhibited the α7 agonist-induced weight loss, decreased food intake, and reduction of glucose levels. The findings indicate that α7 nAChRs play an important role in regulating the biological parameters associated with type 2 diabetes and that this regulation involves JAK2/STAT3 signaling pathways.

Although we did not identify the anatomical localization of the α7 nAChRs involved, it is likely that both central and peripheral components contribute to the effects seen. Areas of the hypothalamus, particularly the lateral hypothalamus that regulates appetite, contain both α7 and α4β2 nAChRs (Jo et al., 2002). Evidence suggests that activation of presynaptic α7 nAChRs on GABAergic terminals in the lateral hypothalamus decreases appetite by inhibiting the activity of melanin-concentrating hormone neurons (Jo et al., 2005). It is possible that the decreases we observed in food consumption and weight gain involve activation of CNS α7 nAChRs because TC-7020 is readily accessible to the brain (oral bioavailability in rats, 33%; brain/plasma ratio = 0.4 at 4 h postdose) and the effects of TC-7020 were blocked by the α7 antagonist MLA. Further-

Discussion

In the present study, we have explored the role of α7 nAChRs in regulating key biological pathways involved in type 2 diabetes and probed the potential of selective α7 nAChR agonists as a novel therapeutic approach to treat this condition. The results indicate that a prototypical selective α7 nAChR agonist can reduce the proinflammatory cytokine TNF-α, reduce elevated glucose levels, decrease glycated hemoglobin, reduce triglycerides, and reduce food intake and

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>TC-7020</th>
<th>Control</th>
<th>Compound A (α4β2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/day)</td>
<td>6.1 ± 0.5</td>
<td>3.9 ± 0.4*</td>
<td>6.0 ± 0.9</td>
<td>2.9 ± 0.6*</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>65 ± 9</td>
<td>45 ± 7*</td>
<td>55 ± 9</td>
<td>44 ± 6*</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>401 ± 11</td>
<td>268 ± 14*</td>
<td>344 ± 19</td>
<td>284 ± 15</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/dl)</td>
<td>380 ± 50</td>
<td>140 ± 25*</td>
<td>388 ± 21</td>
<td>411 ± 12</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>42 ± 5</td>
<td>19 ± 2*</td>
<td>43 ± 6</td>
<td>52 ± 2</td>
</tr>
</tbody>
</table>

Obese (db−) mice were administered vehicle (control), TC-7020 (1 mg/kg), or Compound A (3 mg/kg) once daily by oral gavage for 14 days. Results represent the mean ± S.E.M. (n = 8; *p < 0.05).

**Fig. 7.** Effects of the α7 nAChR agonist TC-7020 and JAK2 inhibitor AG-490 on body mass and food consumption. Both lean (db−) and obese (db−) mice were treated for 7 weeks either with vehicle (V) or TC-7020 (TC). In some experiments, obese mice were treated with TC-7020 plus the JAK2 inhibitor AG-490 (AG). TC-7020 (1 mg/kg) was administered daily via oral gavage. AG-490 was administered daily intraperitoneally (1 mg/kg). Results represent the mean ± S.E.M. of eight treated mice and are expressed as their body mass in grams (A) or average food consumption in grams/day (B) at week 7. *p < 0.05, significantly different from lean vehicle controls; #p < 0.05, significantly different from obese vehicle controls; †p > 0.05, not significantly different from obese vehicle controls.

kine TNF-α, and reduction of triglyceride levels. The α4β2 nAChR-selective compound reduced weight gain and food intake but did not elicit significant changes in any of the other parameters.
tion of inflammatory cytokines, including TNF-α (Zeyda and Stulnig, 2007). Increases in TNF-α and concomitant insulin resistance of adipocytes can lead to a cascade of events, including mitochondrial damage, increased lipolysis, and redistribution of fatty acids to ectopic triglyceride deposits (Ruan and Lodish, 2003; Maassen, 2008). Relevant to the present findings, previous studies suggest a direct link between α7 nAChRs and regulation of TNF-α in adipocytes, whereby activation of α7 nAChRs reduces TNF-α protein levels (Liu et al., 2004). This may partly explain the normalization of TNF-α levels by the selective α7 agonist TC-7020.

The effects of TC-7020 on TNF-α are also consistent with the previously reported involvement of peripheral α7 nAChRs in the cholinergic anti-inflammatory pathway (de Jonge and Ulloa, 2007). This pathway involves the vagus nerve, which uses acetylcholine to activate α7 nAChRs on macrophages, leading to decreased production of inflammatory cytokines, including TNF-α by these cells (Gallowitsch-Puerta and Tracey, 2005). Previous studies that examined the effects of nicotine on LPS-treated and control peritoneal macrophages have shown that nicotine treatment leads to phosphorylation of STAT3 and that this nicotine-mediated effect is blocked by the α7-selective antagonists α-bungarotoxin and MLA and by AG-490, a selective inhibitor of JAK2 phosphorylation (de Jonge et al., 2005). Taken together, these data support the interaction of JAK2 and α7 nAChRs in macrophages and reveal the critical role played by STAT3 in mediating peripheral cholinergic anti-inflammatory effects. The present results extend these findings to show the relevance of α7 nAChR interactions with JAK2 in modulating the biological parameters associated with the development of type 2 diabetes, including increased food intake, weight gain, and dyslipidemia. In this regard, α7-mediated regulation of macrophage-derived inflammatory factors in adipose tissue may play a prominent role.

The effects of TC-7020 on blood glucose and glycated hemoglobin levels are somewhat more difficult to interpret. On the one hand, it may simply be a consequence of weight loss, which is known to improve glucose metabolism and insulin sensitivity in obesity and diabetes (Williams et al., 2003). However, this does not explain why the α4β2-selective compound (Compound A) also reduced food consumption and weight gain but did not affect any of the metabolic parameters, including glucose levels. Another possible explanation is that increased insulin sensitivity in adipose and other tissues by α7-mediated reduction of proinflammatory components facilitates more efficient uptake and metabolism of glucose by cells. Additional studies will be required to probe the mechanistic basis of these findings.

Although our studies provide insights into the molecular pathways recruited during the development of type 2 diabetes, the relative participation and contribution of central cholinergic pathways and peripheral mechanisms remain to be fully elucidated. Likewise, there is still an open debate on the causal relationship of insulin resistance and inflammation observed in type 2 diabetes, i.e., is inflammation responsible for the associated insulin resistance or does insulin resistance lead to proinflammatory cascades. Although the present studies did not address this specific question, a reversal of proinflammatory cytokines before decreases in weight gain would indicate a causal linkage between them. Conversely, α7-selective drugs could have a primary effect on food intake leading to decreased weight gain, and this could result in normalization of insulin resistance and decreased inflammatory effects. It is hoped that future studies will give us a better understanding of these mechanisms and ultimately lead to the development of therapies that target specific nAChR receptor subtypes and downstream signaling pathways as a novel approach to the management of type 2 diabetes.

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