TGR5

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Takeda G protein-coupled receptor (TGR5) is a metabolic regulator, which is also involved in inflammatory responses. TGR5 belongs to the G protein-coupled receptor (GPCR) superfamily.

Keywords: metabolic syndrome ; TGR5 ; etanercept ; heart ; urinary bladder

1. Introduction

Takeda G protein-coupled receptor (TGR5) belongs to the G protein-coupled receptor (GPCR) superfamily [1]. In addition to the heart [2], TGR5 is expressed in other organs and is amenable to being targeted by bile acids in both healthy and diseased states [3]. TGR5 is a metabolic regulator, which is also involved in inflammatory responses [4]. TGR5 activation induces cytoprotective changes in the heart [5][6]. At toxic concentrations, bile acid may stimulate cholinergic M2 receptors, which cause negative effects on myocardial contractility and heart rate [7]. Therefore, TGR5 activation is introduced to provide benefits to cardiac function [8]. Recently, it has been documented that cardiac TGR5 expression is promoted in type-1 diabetic rats [9], mainly due to hyperglycemia, which seems to be related to compensatory homeostasis. However, TGR5 expression in other metabolic disorders remains unknown.

Metabolic syndrome (MetS) belongs to a pre-diabetic state and is a prevalent, multifactorial, and complex disorder, associated with a higher risk of developing diabetes and other cardiovascular complications [10]. Management of MetS is required owing to an increase in the global prevalence [11]. Therefore, several animal models mimicking MetS have been developed, and a high-fat diet (HFD) is popularly used to feed animals [12]. Lipid-induced injury, known as lipotoxicity, is mainly associated with hyperlipidemia, a condition caused by a HFD [13]. Chronic inflammation is known to be linked to hyperlipidemia, causing the induction of cardiovascular diseases (CVDs) [14], because inflammation due to lipid accumulation and excess lipids may have an effect on cell membranes [15]. Therefore, the inflammation caused by a HFD is associated with metabolic disorders that have been observed in rats [12] and mice [16]. From animal studies, a HFD is known to trigger acute and/or chronic inflammation through a complex mechanism [17], with inflammation being mentioned as an important factor for changes in cardiac TGR5 expression [6].

Generally, inflammation is associated with an increase in the levels of plasma cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) [18]. These inflammatory markers have been widely identified in HFD-fed animals, such as cardiac inflammation observed in HFD-fed animals [19], with a higher expression of TNF-α [20]. TNF-α is known to be involved in cardiac injury, acting through inflammatory pathways and/or the activation of cell death programming, including apoptosis [21]. In a clinical setting, TNF-α inhibitors are widely used to treat TNF-α-associated disorders, including rheumatoid arthritis, psoriasis, ankylosing spondylitis, Crohn's disease, diabetes mellitus, Alzheimer's disease, and cancer [22]. These drugs, named anti-TNF agents, are mostly prepared from monoclonal antibodies, such as adalimumab, golimumab, infliximab, and certolizumab pegol, except etanercept, which is a receptor fusion protein [23]. They can suppress responses to TNF-α regardless of the receptor subtype [23]. In animal research, etanercept (Enbrel@), a TNF-α inhibitor [23], can block the activity of TNF-α competitively at an effective dose [23]. Moreover, in addition to heart failure, the overexpression of TNF-α in mice promotes the occurrence of cardiac hypertrophy [24]. It has been documented that etanercept may alleviate cardiac hypertrophy through inhibition of the TNF-α receptor [25].

2. TGR5 expression in the heart or urinary bladder

First, we confirmed the cardiac functional response to TGR5 using the Langendorff apparatus. In the hearts isolated from normal rats, LCA enhanced cardiac contractility and decreased the heart rate due to TGR5 activation. TGR5 transduces signals through Gs protein-mediated cAMP accumulation and can modulate cardiac functions [1]. Moreover, the LCA-induced increase in contractility was more marked in the hearts isolated from HFD-fed rats than in hearts isolated from normal rats, indicating the increased sensitivity of TGR5 in the hearts of HFD-fed rats. However, spontaneous contractility

was found to be reduced in the hearts isolated from HFD-fed rats compared to those isolated from normal rats. This result may be due to the reduced spontaneous contractility in HFD-fed rats. Moreover, we found that cardiac TGR5 expression truly increased in the hearts of HFD-fed rats at the protein and mRNA levels using Western blotting analysis and qPCR, respectively. Therefore, cardiac TGR5 expression was identified to be promoted in HFD-fed rats.

High-fat consumption is specifically known to be a causal factor in the development of cardiac damage [26]. The reductions in spontaneous contractility in HFD-fed rat hearts are consistent with this view. Cardiac damage is an inflammatory injury dependent on oxidative stress [27]. Additionally, a HFD induces insulin resistance and increases TNF-α expression. TNF-α promotes neutrophil-mediated tissue injury and amplifies inflammatory cascades by activating macrophages and other types of cells [28]. Functionally, TNF-α exerts a negative inotropic effect to inhibit myocardial contractility and lower blood pressure [29]. In the present study, the plasma levels of TNF-α and other cytokines markedly increased in HFD-fed rats. Moreover, we used etanercept at an effective dose to inhibit TNF-α in rats [23] as the negative control. The different changes in HFD-fed rats receiving etanercept may indicate the role of TNF-α. Notably, the changes in HFD-fed rat hearts were reversed by etanercept, as determined by a Langendorff assay. Therefore, the cardiac injury induced in HFD-fed rats seems to be associated with TNF-α, which is consistent with the findings of a previous report [30]. Moreover, in the current study, etanercept reversed cardiac TGR5 expression at both the protein and mRNA levels in HFD-fed rats.

STAT3 is a cytoplasmic transcription factor that transmits extracellular signals to the nucleus [31]. Activated STAT3 in the nucleus binds to specific DNA promoter sequences to regulate gene expression [32]. In the current study, cardiac TGR5 expression was promoted in parallel with STAT3 activation. Interestingly, etanercept also inhibited the activation of STAT3 in HFD-fed rat hearts. TNF-α inhibitors, etanercept, and adalimumab can downregulate p-STAT3 expression in human Th17-polarized cells [26]. STAT3 activation provides an important link between inflammation and cardiac fibrosis [27]. STAT3 accumulation in the nucleus can increase the expression of pro-inflammatory cytokine IL-6, which is involved in the pathogenesis of various chronic inflammatory diseases [28]. In the current study, plasma TNF-α and IL-6 levels that increased in HFD-fed rats were also found to be reduced by etanercept. Therefore, etanercept-mediated inhibition of TNF-α may result in downregulation of the IL-6/JAK/STAT3 pathway in HFD-fed rats. Additionally, STAT3 accumulation in the nucleus can also induce the expression of IL-6 and other proinflammatory genes [33]. Moreover, TNF-α can induce cardiac apoptosis, which is also involved in ventricular remodeling [30]. Therefore, the changes in TGR5 expression need to be investigated further.

Inflammation increases STAT3 activation, which contributes to the pathophysiology of tissue injury [34]. STAT3 activation and an increase in the ratio of phosphorylated STAT3 (p-STAT3) to STAT3 may promote nuclear translocation. Moreover, STAT3 was phosphorylated at Y705 and S727 in cells during cytokine-induced STAT3 activation [35]. Therefore, in the current study, we focused on changes in the ratio of p-STAT3 to STAT3, which is indicative of STAT3 activation. Interestingly, STAT3 activation was enhanced, along with the promotion of TGR5 expression in the heart. Our data also demonstrated that the increased ratio of p-STAT3 to STAT3 was reversed by etanercept in the hearts of HFD-fed rats. Mediation of STAT3 activation in terms of increased expression of cardiac TGR5 in HFD-fed rats can thus be considered.

Additionally, to understand whether or not the increased TGR5 expression was specific to the heart, we used female rats to investigate the changes in the urinary bladder, as described previously [36]. The changes were the same as those observed in the hearts. TGR5 expression in the urinary bladder was reduced in HFD-fed rats, as shown in a cystometrogram. This effect was reversed by etanercept at an effective dose to inhibit TNF-α in rats [22], indicating the role of cytokines in changes in the urinary bladder of HFD-fed rats. The high expression of TGR5 in urinary bladders was also a characteristic feature in these rats. We used betulinic acid to replace LCA for the activation of TGR5 in the urinary bladder. Betulinic acid is a natural triterpene that has been demonstrated to activate TGR5 [37]. Notably, relaxation in the urinary bladder by betulinic acid was more marked in HFD-fed rats than in normal rats, as determined from a cystometrogram. This view was supported by the increased mRNA levels of TGR5 in urinary bladders isolated from HFD-fed rats. Moreover, TGR5 is a member of the family of GPCRs that may increase cAMP levels [1]. We found that betulinic acid induced an increase in cAMP more markedly in the urinary bladders isolated from HFD-fed rats than in those isolated from normal rats. Therefore, TGR5 expression is increased in the urinary bladder during metabolic disorders. An increase in TGR5 expression could be a compensatory response against the lipotoxicity observed in HFD-mediated damage. However, this hypothesis needs further investigation in the future. The main limitation of this study is that the effect of etanercept in normal rats was not compared. The relationships between etanercept and TGR5 expression in a MetS model require further investigation.

3. Conclusions

TGR5 expression is elevated in the heart or urinary bladder of HFD-fed rats. Notably, etanercept is effective in ameliorating inflammation and decreases TGR5 expression in HFD-induced obese rats. These results have implications for dysfunction in the heart or urinary bladder, particularly the association between inflammatory cytokines and TGR5 activation, which provides the benefit of reversing the dysfunction. The development of tissue-specific drugs that target TGR5 expression could provide benefits for assessing interventions in metabolic disease.

References

1. Tiwari, A.; Maiti, P. TGR5: An emerging bile acid G-protein-coupled receptor target for the potential treatment of metabolic disorders. Drug Discov. Today 2009, 14, 523–530.
2. Desai, M.S.; Shabier, Z.; Taylor, M.; Lam, F.; Thevananther, S.; Kosters, A.; Karpen, S.J. Hypertrophic cardiomyopathy and dysregulation of cardiac energetics in a mouse model of biliary fibrosis. Hepatology 2010, 51, 2097–2107.
3. Desai, M.S.; Penny, D.J. Bile acids induce arrhythmias: Old metabolite, new tricks. Heart 2013, 99, 1629–1630.
4. Guo, C.; Chen, W.D.; Wang, Y.D. TGR5, Not Only a Metabolic Regulator. Front. Physiol. 2016, 7, 646.
5. Eblimit, Z.; Thevananther, S.; Karpen, S.J.; Taegtmeyer, H.; Moore, D.D.; Adorini, L.; Penny, D.J.; Desai, M.S. TGR5 activation induces cytoprotective changes in the heart and improves myocardial adaptability to physiologic, inotropic, and pressure-induced stress in mice. Cardiovasc. Ther. 2018, 36, e12462.
6. Li, J.; Cheng, R.; Wan, H. Overexpression of TGR5 alleviates myocardial ischemia/reperfusion injury via AKT/GSK-3beta mediated inflammation and mitochondrial pathway. Biosci. Rep. 2020, 40, BSR20193482.
7. Sheikh Abdul Kadir, S.H.; Miragoli, M.; Abu-Hayyeh, S.; Moshkov, A.V.; Xie, Q.; Keitel, V.; Nikolaev, V.O.; Williamson, C.; Gorelik, J. Bile acid-induced arrhythmia is mediated by muscarinic M2 receptors in neonatal rat cardiomyocytes. PLoS ONE 2010, 5, e9689.
8. Cheng, K.C.; Chang, W.T.; Kuo, F.Y.; Chen, Z.C.; Li, Y.; Cheng, J.T. TGR5 activation ameliorates hyperglycemia-induced cardiac hypertrophy in H9c2 cells. Sci. Rep. 2019, 9, 3633.
9. Kuo, Y.F.; Lee, S.P.; Cheng, J.T.; Wu, M.C. Cardiac TGR5 expression enhanced by hyperglycaemia in diabetic rats: A preclinical warning for disorders with excess bile acids. Integr. Mol. Med. 2019, 6, 1–7.
10. O'Neill, S.; O'Driscoll, L. Metabolic syndrome: A closer look at the growing epidemic and its associated pathologies. Obes. Rev. 2015, 16, 1–12.
11. Nolan, P.B.; Carrick-Ranson, G.; Stinear, J.W.; Reading, S.A.; Dalleck, L.C. Prevalence of metabolic syndrome and metabolic syndrome components in young adults: A pooled analysis. Prev. Med. Rep. 2017, 7, 211–215.
12. Axen, K.V.; Dikeakos, A.; Sclafani, A. High dietary fat promotes syndrome X in nonobese rats. J. Nutr. 2003, 133, 2244–2249.
13. Chan, R.S.; Woo, J. Prevention of overweight and obesity: How effective is the current public health approach. Int. J. Environ. Res. Public Health 2010, 7, 765–783.
14. Dai, S.; Huang, B.; Zou, Y.; Liu, Y. Associations of dipping and non-dipping hypertension with cardiovascular diseases in patients with dyslipidemia. Arch. Med. Sci. 2019, 15, 337–342.
15. Rocha, V.Z.; Libby, P. Obesity, inflammation, and atherosclerosis. Nat. Rev. Cardiol. 2009, 6, 399–409.
16. Ma, Y.; Gao, M.; Liu, D. Chlorogenic acid improves high fat diet-induced hepatic steatosis and insulin resistance in mice. Pharm. Res. 2015, 32, 1200–1209.
17. Chen, X.; Yu, W.; Li, W.; Zhang, H.; Huang, W.; Wang, J.; Zhu, W.; Fang, Q.; Chen, C.; Li, X.; et al. An anti-inflammatory chalcone derivative prevents heart and kidney from hyperlipidemia-induced injuries by attenuating inflammation. Toxicol. Appl. Pharmacol. 2018, 338, 43–53.
18. Das, A.; Mukhopadhyay, S. The evil axis of obesity, inflammation and type-2 diabetes. Endocr. Metab. Immune Disord. Drug Targets 2011, 11, 23–31.
19. Chen, Y.; Feng, B.; Yuan, Y.; Hu, J.; Zhao, W.; Jiang, H.; Li, W.; Fan, Z.; Du, Z. Aloe Emodin Reduces Cardiac Inflammation Induced by a High-Fat Diet through the TLR4 Signaling Pathway. Mediat. Inflamm. 2020, 2020, 6318520.
20. Borst, S.E.; Conover, C.F. High-fat diet induces increased tissue expression of TNF-alpha. Life Sci. 2005, 77, 2156–2165.

21. Chytilova, A.; Borchert, G.H.; Mandikova-Alanova, P.; Hlavackova, M.; Kopkan, L.; Khan, M.A.; Imig, J.D.; Kolar, F.; Neckar, J. Tumour necrosis factor-alpha contributes to improved cardiac ischaemic tolerance in rats adapted to chronic continuous hypoxia. Acta Physiol. 2015, 214, 97–108.
22. Balkwill, F. Tumour necrosis factor and cancer. Nat. Rev. Cancer 2009, 9, 361–371.
23. Murdaca, G.; Spano, F.; Contatore, M.; Guastalla, A.; Magnani, O.; Puppo, F. Pharmacogenetics of etanercept: Role of TNF-alpha gene polymorphisms in improving its efficacy. Expert Opin. Drug Metab. Toxicol. 2014, 10, 1703–1710.
24. Hartupee, J.; Szalai, G.D.; Wang, W.; Ma, X.; Diwan, A.; Mann, D.L. Impaired Protein Quality Control During Left Ventricular Remodeling in Mice With Cardiac Restricted Overexpression of Tumor Necrosis Factor. Circ. Heart Fail. 2017, 10, e004252.
25. Li, Q.; Yu, Q.; Na, R.; Liu, B. Etanercept protects rat cardiomyocytes against hypertrophy by regulating inflammatory cytokines secretion and cell apoptosis. Braz. J. Med. Biol. Res. 2017, 50, e5868.
26. Cole, M.A.; Murray, A.J.; Cochlin, L.E.; Heather, L.C.; McAleese, S.; Knight, N.S.; Sutton, E.; Jamil, A.A.; Parassol, N.; Clarke, K. A high fat diet increases mitochondrial fatty acid oxidation and uncoupling to decrease efficiency in rat heart. Basic Res. Cardiol. 2011, 106, 447–457.
27. Yida, Z.; Imam, M.U.; Ismail, M.; Ismail, N.; Ideris, A.; Abdullah, M.A. High fat diet-induced inflammation and oxidative stress are attenuated by N-acetylneuraminic acid in rats. J. Biomed. Sci. 2015, 22, 96.
28. Speyer, C.L.; Ward, P.A. Role of endothelial chemokines and their receptors during inflammation. J. Investig. Surg. 2011, 24, 18–27.
29. Prabhu, S.D. Cytokine-induced modulation of cardiac function. Circ. Res. 2004, 95, 1140–1153.
30. Zhu, J.; Liu, M.; Kennedy, R.H.; Liu, S.J. TNF-alpha-induced impairment of mitochondrial integrity and apoptosis mediated by caspase-8 in adult ventricular myocytes. Cytokine 2006, 34, 96–105.
31. Miller, A.M.; Wang, H.; Bertola, A.; Park, O.; Horiguchi, N.; Ki, S.H.; Yin, S.; Lafdil, F.; Gao, B. Inflammation-associated interleukin-6/signal transducer and activator of transcription 3 activation ameliorates alcoholic and nonalcoholic fatty liver diseases in interleukin-10-deficient mice. Hepatology 2011, 54, 846–856.
32. Jung, J.E.; Lee, H.G.; Cho, I.H.; Chung, D.H.; Yoon, S.H.; Yang, Y.M.; Lee, J.W.; Choi, S.; Park, J.W.; Ye, S.K.; et al. STAT3 is a potential modulator of HIF-1-mediated VEGF expression in human renal carcinoma cells. FASEB J. 2005, 19, 1296–1298.
33. Zgheib, C.; Zouein, F.A.; Kurdi, M.; Booz, G.W. Differential STAT3 signaling in the heart: Impact of concurrent signals and oxidative stress. JAKSTAT 2012, 1, 101–110.
34. Bharadwaj, U.; Kasembeli, M.M.; Robinson, P.; Tweardy, D.J. Targeting Janus Kinases and Signal Transducer and Activator of Transcription 3 to Treat Inflammation, Fibrosis, and Cancer: Rationale, Progress, and Caution. Pharmacol. Rev. 2020, 72, 486–526.
35. Mori, T.; Miyamoto, T.; Yoshida, H.; Asakawa, M.; Kawasumi, M.; Kobayashi, T.; Morioka, H.; Chiba, K.; Toyama, Y.; Yoshimura, A. IL-1beta and TNFalpha-initiated IL-6-STAT3 pathway is critical in mediating inflammatory cytokines and RANKL expression in inflammatory arthritis. Int. Immunol. 2011, 23, 701–712.
36. Liu, K.M.; Chuang, S.M.; Long, C.Y.; Lee, Y.L.; Wang, C.C.; Lu, M.C.; Lin, R.J.; Lu, J.H.; Jang, M.Y.; Wu, W.J.; et al. Ketamine-induced ulcerative cystitis and bladder apoptosis involve oxidative stress mediated by mitochondria and the endoplasmic reticulum. Am. J. Physiol. Renal. Physiol. 2015, 309, F318–F331.
37. Lo, S.H.; Cheng, K.C.; Li, Y.X.; Chang, C.H.; Cheng, J.T.; Lee, K.S. Development of betulinic acid as an agonist of TGR5 receptor using a new in vitro assay. Drug Des. Devel. Ther. 2016, 10, 2669–2676.