17. Dunbar JC, Hu Y, Lu H. Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes.* 1997;46:2040–2043.
18. Shek EW, Brands MW, Hall JE. Chronic leptin infusion increases arterial pressure. *Hypertension.* 1998;31:409–414.
19. Correia ML, Morgan DA, Sivitz WI, Mark AL, Haynes WG. Leptin acts in the central nervous system to produce dose-dependent changes in arterial pressure. *Hypertension.* 2001;37:936–942.
20. Aizawa-Abe M, Ogawa Y, Masuzaki H, Ebihara K, Satoh N, Iwai H, Matsuoka N, Hayashi T, Hosoda K, Inoue G, Yoshimasa Y, Nakao K. Pathophysiological role of leptin in obesity-related hypertension. *J Clin Invest.* 2000;105:1243–1252.
21. Mark AL, Shaffer RA, Correia ML, Morgan DA, Sigmund CD, Haynes WG. Contrasting blood pressure effects of obesity in leptin-deficient ob/ob mice and agouti yellow obese mice. *J Hypertens.* 1999;17: 1949–1953.
22. Agata J, Masuda A, Takada M, Higashiura K, Murakami H, Miyazaki Y, Shimamoto K. High plasma immunoreactive leptin level in essential hypertension. *Am J Hypertens.* 1997;10:1171–1174.
23. Eikelis N, Schlaich M, Aggarwal A, Kaye D, Esler M. Interactions between leptin and the human sympathetic nervous system. *Hypertension.* 2003;41: 1072–1079.
24. Mackintosh RM, Hirsch J. The effects of leptin administration in nonobese human subjects. *Obes Res.* 2001;9:462–469.
25. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, McCamish M. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA.* 1999;282:1568–1575.
26. Correia ML, Rahmouni K. Role of leptin in the cardiovascular and endocrine complications of metabolic syndrome. *Diabetes Obes Metab.* 2006;8:603–610.
27. Rahmouni K, Morgan DA, Morgan GM, Mark AL, Haynes WG. Role of selective leptin resistance in diet-induced obesity hypertension. *Diabetes.* 2005;54:2012–2018.
28. Ciccone M, Vettor R, Pannacciulli N, Minenna A, Bellacicco M, Rizzon P, Giorgino R, De Pergola G. Plasma leptin is independently associated with the intima-media thickness of the common carotid artery. *Int J Obes Relat Metab Disord.* 2001;25:805–810.
29. Singhal A, Farooqi IS, Cole TJ, O'Rahilly S, Fewtrell M, Kattenhorn M, Lucas A, Deanfield J. Influence of leptin on arterial distensibility: a novel link between obesity and cardiovascular disease? *Circulation.* 2002;106:1919–1924.
30. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, Sattar N. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). *Circulation.* 2001; 104:3052–3056.
31. Wolk R, Berger P, Lennon RJ, Brilakis ES, Johnson BD, Somers VK. Plasma leptin and prognosis in patients with established coronary atherosclerosis. *J Am Coll Cardiol.* 2004;44:1819–1824.
32. Schafer K, Halle M, Goeschen C, Dellas C, Pynn M, Loskutoff DJ, Konstantinides S. Leptin promotes vascular remodeling and neointimal growth in mice. *Arterioscler Thromb Vasc Biol.* 2004;24:112–117.
33. Oda A, Taniguchi T, Yokoyama M. Leptin stimulates rat aortic smooth muscle cell proliferation and migration. *Kobe J Med Sci.* 2001;47: 141–150.
34. Stephenson K, Tunstead J, Tsai A, Gordon R, Henderson S, Dansky HM. Neointimal formation after endovascular arterial injury is markedly attenuated in db/db mice. *Arterioscler Thromb Vasc Biol.* 2003;23: 2027–2033.
35. Cleland SJ, Sattar N, Petrie JR, Forouhi NG, Elliott HL, Connell JM. Endothelial dysfunction as a possible link between C-reactive protein levels and cardiovascular disease. *Clin Sci (Lond).* 2000;98:531–535.
36. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD, Diehl AM. Leptin regulates proinflammatory immune responses. *FASEB J.* 1998;12: 57–65.
37. Kazumi T, Kawaguchi A, Hirano T, Yoshino G. C-reactive protein in young, apparently healthy men: associations with serum leptin, QTc interval, and high-density lipoprotein-cholesterol. *Metabolism.* 2003;52: 1113–1116.
38. Konstantinides S, Schafer K, Koschnick S, Loskutoff DJ. Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity. *J Clin Invest.* 2001; 108:1533–1540.
39. Bodary PF, Westrick RJ, Wickenheiser KJ, Shen Y, Eitzman DT. Effect of leptin on arterial thrombosis following vascular injury in mice. *JAMA.* 2002;287:1706–1709.
40. Maruyama I, Nakata M, Yamaji K. Effect of leptin in platelet and endothelial cells. Obesity and arterial thrombosis. *Ann N Y Acad Sci.* 2000;902:315–319.
41. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest.* 2004;114:1752–1761.
42. Yamagishi SI, Edelstein D, Du XL, Kaneda Y, Guzman M, Brownlee M. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. *J Biol Chem.* 2001;276:25096–25100.
43. Beltowski J, Wojcicka G, Jamroz A. Leptin decreases plasma paraoxonase 1 (PON1) activity and induces oxidative stress: the possible novel mechanism for proatherogenic effect of chronic hyperleptinemia. *Atherosclerosis.* 2003;170:21–29.
44. Maingrette F, Renier G. Leptin increases lipoprotein lipase secretion by macrophages: involvement of oxidative stress and protein kinase C. *Diabetes.* 2003;52:2121–2128.
45. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem.* 1995;270:26746–26749.
46. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun.* 1996;221:286–289.
47. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem.* 1996;271:10697–10703.
48. Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M. Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *J Biochem (Tokyo).* 1996;120:803–812.
49. Shapiro L, Scherer PE. The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor. *Curr Biol.* 1998;8:335–338.
50. Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. *J Biol Chem.* 2003;278:9073–9085.
51. Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. *J Biol Chem.* 2003;278:40352–40363.
52. Takahashi M, Funahashi T, Shimomura I, Miyaoka K, Matsuzawa Y. Plasma leptin levels and body fat distribution. *Horm Metab Res.* 1996; 28:751–752.
53. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun.* 1999; 257:79–83.
54. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia.* 2003;46:459–469.
55. Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, Nagai M, Matsuzawa Y, Funahashi T. Adiponectin as a biomarker of the metabolic syndrome. *Circ J.* 2004;68:975–981.
56. Halleux CM, Takahashi M, Delporte ML, Detry R, Funahashi T, Matsuzawa Y, Brichard SM. Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. *Biochem Biophys Res Commun.* 2001;288:1102–1107.
57. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes.* 2001;50:2094–2099.
58. Ran J, Hirano T, Fukui T, Saito K, Kageyama H, Okada K, Adachi M. Angiotensin II infusion decreases plasma adiponectin level via its type

1 receptor in rats: an implication for hypertension-related insulin resistance. *Metabolism.* 2006;55:478–488.
59. Delporte ML, Funahashi T, Takahashi M, Matsuzawa Y, Brichard SM. Pre- and post-translational negative effect of beta-adrenoceptor agonists on adiponectin secretion: in vitro and in vivo studies. *Biochem J.* 2002;367:677–685.
60. Imai J, Katagiri H, Yamada T, Ishigaki Y, Ogihara T, Uno K, Hasegawa Y, Gao J, Ishihara H, Sasano H, Oka Y. Cold exposure suppresses serum adiponectin levels through sympathetic nerve activation in mice. *Obesity (Silver Spring).* 2006;14:1132–1141.
61. Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF. T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. *Proc Natl Acad Sci U S A.* 2004;101:10308–10313.
62. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature.* 2003;423:762–769.
63. Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, Tsuchida A, Kumagai K, Kozono H, Hada Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Awazawa M, Takamoto I, Froguel P, Hara K, Tobe K, Nagai R, Ueki K, Kadowaki T. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med.* 2007;33:332–339.
64. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest.* 2006;116:1784–1792.
65. Adamczak M, Wiecek A, Funahashi T, Chudek J, Kokot F, Matsuzawa Y. Decreased plasma adiponectin concentration in patients with essential hypertension. *Am J Hypertens.* 2003;16:72–75.
66. Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, Kumada M, Ohashi K, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Nagasawa A, Kobayashi H, Hiraoka H, Komai N, Kaibe M, Rakugi H, Ogihara T, Matsuzawa Y. Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension.* 2003;42:231–234.
67. Furuhashi M, Ura N, Higashiura K, Murakami H, Tanaka M, Moniwa N, Yoshida D, Shimamoto K. Blockade of the renin-angiotensin system increases adiponectin concentrations in patients with essential hypertension. *Hypertension.* 2003;42:76–81.
68. Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, Ohashi K, Sakai N, Shimomura I, Kobayashi H, Terasaka N, Inaba T, Funahashi T, Matsuzawa Y. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation.* 2002;106:2767–2770.
69. Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, Uchida S, Ito Y, Takakuwa K, Matsui J, Takata M, Eto K, Terauchi Y, Komeda K, Tsunoda M, Murakami K, Ohnishi Y, Naitoh T, Yamamura K, Ueyama Y, Froguel P, Kimura S, Nagai R, Kadowaki T. Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem.* 2003;278:2461–2468.
70. Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S, Kadowaki T, Noda T. Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem.* 2002;277:25863–25866.
71. Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, Kumada M, Okamoto Y, Nagaretani H, Nishizawa H, Kishida K, Komuro R, Ouchi N, Kihara S, Nagai R, Funahashi T, Matsuzawa Y. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *J Biol Chem.* 2002;277:37487–37491.
72. Ohashi K, Ouchi N, Kihara S, Funahashi T, Nakamura T, Sumitsuji S, Kawamoto T, Matsumoto S, Nagaretani H, Kumada M, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Hiraoka H, Iwashima Y, Ishikawa K, Ohishi M, Katsuya T, Rakugi H, Ogihara T, Matsuzawa Y. Adiponectin I164T mutation is associated with the metabolic syndrome and coronary artery disease. *J Am Coll Cardiol.* 2004;43:1195–1200.
73. Filippi E, Sentinelli F, Romeo S, Arca M, Berni A, Tiberti C, Verrienti A, Fanelli M, Fallarino M, Sorropago G, Baroni MG. The adiponectin gene SNP+276G>T associates with early-onset coronary artery disease and with lower levels of adiponectin in younger coronary artery disease patients (age <or=50 years). *J Mol Med.* 2005;83:711–719.
74. Combs TP, Wagner JA, Berger J, Doebber T, Wang WJ, Zhang BB, Tanen M, Berg AH, O'Rahilly S, Savage DB, Chatterjee K, Weiss S, Larson PJ, Gottesdiener KM, Gertz BJ, Charron MJ, Scherer PE, Moller DE. Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization. *Endocrinology.* 2002;143:998–1007.
75. Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefebvre PJ, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge J, Birkeland K, Golay A, Heine RJ, Koranyi L, Laakso M, Mokan M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W, Schernthaner G, Schmitz O, Skrha J, Smith U, Taton J. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet.* 2005;366:1279–1289.
76. Nawrocki AR, Rajala MW, Tomas E, Pajvani UB, Saha AK, Trumbauer ME, Pang Z, Chen AS, Ruderman NB, Chen H, Rossetti L, Scherer PE. Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. *J Biol Chem.* 2006;281:2654–2660.
77. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem.* 2003;278:45021–45026.
78. Hattori Y, Suzuki M, Hattori S, Kasai K. Globular adiponectin upregulates nitric oxide production in vascular endothelial cells. *Diabetologia.* 2003;46:1543–1549.
79. Motoshima H, Wu X, Mahadev K, Goldstein BJ. Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. *Biochem Biophys Res Commun.* 2004;315:264–271.
80. Kobayashi H, Ouchi N, Kihara S, Walsh K, Kumada M, Abe Y, Funahashi T, Matsuzawa Y. Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circ Res.* 2004;94:e27–e31.
81. Lin LY, Lin CY, Su TC, Liau CS. Angiotensin II-induced apoptosis in human endothelial cells is inhibited by adiponectin through restoration of the association between endothelial nitric oxide synthase and heat shock protein 90. *FEBS Lett.* 2004;574:106–110.
82. Kobashi C, Urakaze M, Kishida M, Kibayashi E, Kobayashi H, Kihara S, Funahashi T, Takata M, Temaru R, Sato A, Yamazaki K, Nakamura N, Kobayashi M. Adiponectin inhibits endothelial synthesis of interleukin-8. *Circ Res.* 2005;97:1245–1252.
83. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation.* 2000;102:1296–1301.
84. Wulster-Radcliffe MC, Ajuwon KM, Wang J, Christian JA, Spurlock ME. Adiponectin differentially regulates cytokines in porcine macrophages. *Biochem Biophys Res Commun.* 2004;316:924–929.
85. Yamaguchi N, Argueta JG, Masuhiro Y, Kagishita M, Nonaka K, Saito T, Hanazawa S, Yamashita Y. Adiponectin inhibits Toll-like receptor family-induced signaling. *FEBS Lett.* 2005;579:6821–6826.
86. Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation.* 2001;103:1057–1063.
87. Furukawa K, Hori M, Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Miyazaki A, Nakayama H, Horiuchi S. Adiponectin down-regulates acyl-coenzyme A:cholesterol acyltransferase-1 in cultured human monocyte-derived macrophages. *Biochem Biophys Res Commun.* 2004;317:831–836.
88. Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N, Nagasawa A, Funahashi T, Matsuzawa Y. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation.* 2004;109:2046–2049.
89. Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, Kumada M, Hotta K, Nishida M, Takahashi M, Nakamura T, Shimomura I, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation.* 2002;105:2893–2898.

90. Wang Y, Lam KS, Xu JY, Lu G, Xu LY, Cooper GJ, Xu A. Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner. *J Biol Chem.* 2005;280:18341–18347.
91. Kato H, Kashiwagi H, Shiraga M, Tadokoro S, Kamae T, Ujiie H, Honda S, Miyata S, Ijiri Y, Yamamoto J, Maeda N, Funahashi T, Kurata Y, Shimomura I, Tomiyama Y, Kanakura Y. Adiponectin acts as an endogenous antithrombotic factor. *Arterioscler Thromb Vasc Biol.* 2006;26:224–230.
92. Wolk R, Berger P, Lennon RJ, Brilakis ES, Somers VK. Body mass index: a risk factor for unstable angina and myocardial infarction in patients with angiographically confirmed coronary artery disease. *Circulation.* 2003;108:2206–2211.
93. Orlander PR, Goff DC, Morrissey M, Ramsey DJ, Wear ML, Labarthe DR, Nichaman MZ. The relation of diabetes to the severity of acute myocardial infarction and post-myocardial infarction survival in Mexican-Americans and non-Hispanic whites. The Corpus Christi Heart Project. *Diabetes.* 1994;43:897–902.
94. Shibata R, Sato K, Pimentel DR, Takemura Y, Kihara S, Ohashi K, Funahashi T, Ouchi N, Walsh K. Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nat Med.* 2005;11:1096–1103.
95. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science.* 1993;259:87–91.
96. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature.* 1997;389:610–614.
97. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest.* 2003;112:1796–1808.
98. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest.* 2003;112:1821–1830.
99. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, Yamashita S, Miura M, Fukuda Y, Takemura K, Tokunaga K, Matsuzawa Y. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nat Med.* 1996;2:800–803.
100. Cigolini M, Targher G, Bergamo Andreis IA, Tonoli M, Agostino G, De Sandre G. Visceral fat accumulation and its relation to plasma hemostatic factors in healthy men. *Arterioscler Thromb Vasc Biol.* 1996;16:368–374.
101. Shepherd PR, Kahn BB. Glucose transporters and insulin action–implications for insulin resistance and diabetes mellitus. *N Engl J Med.* 1999;341:248–257.
102. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature.* 2001;409:729–733.
103. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature.* 2005;436:356–362.
104. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U, Kahn BB. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med.* 2006;354:2552–2563.
105. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. *Nature.* 2001;409:307–312.
106. Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, Mickle DA. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. *Circulation.* 2003;108:736–740.
107. Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, Manabe I, Utsunomiya K, Nagai R. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-endothelial cell interactions. *Biochem Biophys Res Commun.* 2004;314:415–419.
108. Nagaev I, Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. *Biochem Biophys Res Commun.* 2001;285:561–564.
109. Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, O'Rahilly S. Resistin / Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. *Diabetes.* 2001;50:2199–2202.
110. Curat CA, Wegner V, Sengenes C, Miranville A, Tonus C, Busse R, Bouloumie A. Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. *Diabetologia.* 2006;49:744–747.
111. Inaba T, Matsuda M, Shimamura M, Takei N, Terasaka N, Ando Y, Yasumo H, Koishi R, Makishima M, Shimomura I. Angiopoietin-like protein 3 mediates hypertriglyceridemia induced by the liver X receptor. *J Biol Chem.* 2003;278:21344–21351.
112. Koishi R, Ando Y, Ono M, Shimamura M, Yasumo H, Fujiwara T, Horikoshi H, Furukawa H. Angptl3 regulates lipid metabolism in mice. *Nat Genet.* 2002;30:151–157.
113. Shimizugawa T, Ono M, Shimamura M, Yoshida K, Ando Y, Koishi R, Ueda K, Inaba T, Minekura H, Kohama T, Furukawa H. ANGPTL3 decreases very low density lipoprotein triglyceride clearance by inhibition of lipoprotein lipase. *J Biol Chem.* 2002;277:33742–33748.
114. Shimamura M, Matsuda M, Kobayashi S, Ando Y, Ono M, Koishi R, Furukawa H, Makishima M, Shimomura I. Angiopoietin-like protein 3, a hepatic secretory factor, activates lipolysis in adipocytes. *Biochem Biophys Res Commun.* 2003;301:604–609.
115. Oike Y, Akao M, Yasunaga K, Yamauchi T, Morisada T, Ito Y, Urano T, Kimura Y, Kubota Y, Maekawa H, Miyamoto T, Miyata K, Matsumoto S, Sakai J, Nakagata N, Takeya M, Koseki H, Ogawa Y, Kadowaki T, Suda T. Angiopoietin-related growth factor antagonizes obesity and insulin resistance. *Nat Med.* 2005;11:400–408.
116. Kersten S, Mandard S, Tan NS, Escher P, Metzger D, Chambon P, Gonzalez FJ, Desvergne B, Wahli W. Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. *J Biol Chem.* 2000;275:28488–28493.
117. Yoon JC, Chickering TW, Rosen ED, Dussault B, Qin Y, Soukas A, Friedman JM, Holmes WE, Spiegelman BM. Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. *Mol Cell Biol.* 2000;20:5343–5349.
118. Xu A, Lam MC, Chan KW, Wang Y, Zhang J, Hoo RL, Xu JY, Chen B, Chow WS, Tso AW, Lam KS. Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. *Proc Natl Acad Sci U S A.* 2005;102:6086–6091.
119. Bartness TJ, Kay Song C, Shi H, Bowers RR, Foster MT. Brain-adipose tissue cross talk. *Proc Nutr Soc.* 2005;64:53–64.
120. Niijima A. Afferent signals from leptin sensors in the white adipose tissue of the epididymis, and their reflex effect in the rat. *J Auton Nerv Syst.* 1998;73:19–25.
121. Tanida M, Iwashita S, Ootsuka Y, Terui N, Suzuki M. Leptin injection into white adipose tissue elevates renal sympathetic nerve activity dose-dependently through the afferent nerves pathway in rats. *Neurosci Lett.* 2000;293:107–110.
122. Yamada T, Katagiri H, Ishigaki Y, Ogihara T, Imai J, Uno K, Hasegawa Y, Gao J, Ishihara H, Niijima A, Mano H, Aburatani H, Asano T, Oka Y. Signals from intra-abdominal fat modulate insulin and leptin sensitivity through different mechanisms: neuronal involvement in food-intake regulation. *Cell Metab.* 2006;3:223–229.
123. Niijima A. Reflex control of the autonomic nervous system activity from the glucose sensors in the liver in normal and midpontine-transected animals. *J Auton Nerv Syst.* 1984;10:279–285.
124. Adachi A, Shimizu N, Oomura Y, Kobashi M. Convergence of hepatoportal glucose-sensitive afferent signals to glucose-sensitive units within the nucleus of the solitary tract. *Neurosci Lett.* 1984;46:215–218.
125. Randich A, Spraggins DS, Cox JE, Meller ST, Kelm GR. Jejunal or portal vein infusions of lipids increase hepatic vagal afferent activity. *Neuroreport.* 2001;12:3101–3105.
126. Benthem L, Keizer K, Wiegman CH, de Boer SF, Strubbe JH, Steffens AB, Kuipers F, Scheurink AJ. Excess portal venous long-chain fatty acids induce syndrome X via HPA axis and sympathetic activation. *Am J Physiol Endocrinol Metab.* 2000;279:E1286–E1293.
127. Grekin RJ, Vollmer AP, Sider RS. Pressor effects of portal venous oleate infusion. A proposed mechanism for obesity hypertension. *Hypertension.* 1995;26:193–198.
128. Grekin RJ, Dumont CJ, Vollmer AP, Watts SW, Webb RC. Mechanisms in the pressor effects of hepatic portal venous fatty acid infusion. *Am J Physiol.* 1997;273:R324–R330.
129. Ishigaki Y, Katagiri H, Yamada T, Ogihara T, Imai J, Uno K, Hasegawa Y, Gao J, Ishihara H, Shimosegawa T, Sakoda H, Asano T, Oka Y. Dissipating excess energy stored in the liver is a potential treatment

strategy for diabetes associated with obesity. *Diabetes*. 2005;54: 322–332.
130. Uno K, Katagiri H, Yamada T, Ishigaki Y, Ogihara T, Imai J, Hasegawa Y, Gao J, Kaneko K, Iwasaki H, Ishihara H, Sasano H, Inukai K, Mizuguchi H, Asano T, Shiota M, Nakazato M, Oka Y. Neuronal pathway from the liver modulates energy expenditure and systemic insulin sensitivity. *Science*. 2006;312:1656–1659.
131. Rahimian R, Masih-Khan E, Lo M, van Breemen C, McManus BM, Dube GP. Hepatic over-expression of peroxisome proliferator activated receptor gamma2 in the ob/ob mouse model of non-insulin dependent diabetes mellitus. *Mol Cell Biochem*. 2001;224:29–37.
132. Chao L, Marcus-Samuels B, Mason MM, Moitra J, Vinson C, Arioglu E, Gavrilova O, Reitman ML. Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. *J Clin Invest*. 2000;106:1221–1228.
133. Matsusue K, Haluzik M, Lambert G, Yim SH, Gavrilova O, Ward JM, Brewer B Jr, Reitman ML, Gonzalez FJ. Liver-specific disruption of PPARgamma in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes. *J Clin Invest*. 2003;111:737–747.
134. Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS. A transgenic model of visceral obesity and the metabolic syndrome. *Science*. 2001;294:2166–2170.
135. Bernal-Mizrachi C, Weng S, Feng C, Finck BN, Knutsen RH, Leone TC, Coleman T, Mecham RP, Kelly DP, Semenkovich CF. Dexamethasone induction of hypertension and diabetes is PPAR-alpha dependent in LDL receptor-null mice. *Nat Med*. 2003;9:1069–1075.
136. Bernal-Mizrachi C, Xiaozhong L, Yin L, Knutsen RH, Howard MJ, Arends JJ, Desantis P, Coleman T, Semenkovich CF. An afferent vagal nerve pathway links hepatic PPARalpha activation to glucocorticoid-induced insulin resistance and hypertension. *Cell Metab*. 2007;5: 91–102.
137. Ahima RS, Qi Y, Singhal NS, Jackson MB, Scherer PE Brain adipocytokine action and metabolic regulation. *Diabetes*. 2006;55(suppl 2): S145–S154.
138. Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, Scherer PE, Ahima RS. Adiponectin acts in the brain to decrease body weight. *Nat Med*. 2004;10:524–529.

Available online at www.sciencedirect.com
ScienceDirect
Pharmacology & Therapeutics 117 (2008) 188–198
Pharmacology & Therapeutics
www.elsevier.com/locate/pharmthera

Associate editor: I. Kimura

Inter-organ metabolic communication involved in energy homeostasis: Potential therapeutic targets for obesity and metabolic syndrome

Tetsuya Yamada [a], Yoshitomo Oka [a], Hideki Katagiri [b,*]

[a] Division of Molecular Metabolism and Diabetes, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
[b] Division of Advanced Therapeutics for Metabolic Diseases, Center for Translational and Advanced Animal Research, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan

Abstract

The global rate of obesity is rising alarmingly, exerting a major adverse impact on human health by increasing the prevalences of disorders, such as diabetes, hypertension and heart disease. To maintain systemic energy homeostasis, metabolic information must be communicated among organs/tissues. Obesity-related disorders can be thought of as resulting from dysregulation of this vital inter-tissue communication. Remarkable advances in obesity research during this decade have shown humoral factors manufactured and secreted by adipose tissue (adipocytokines) to be of great importance. In addition to these humoral factors, such as nutrients (glucose, fatty acids and amino acids) and hormones (insulin, adipocytokines and so on), the functional significance of the autonomic nervous system has recently attracted research attention. Autonomic nerves are essential components of the endogenous system for maintaining energy homeostasis, making them potential therapeutic targets for obesity-related disorders. This review focuses on the therapeutic possibilities of targeting inter-organ communication systems.

Keywords: Obesity; Metabolic syndrome; Inter-organ communication; Energy homeostasis; Autonomic nervous system; Central nervous system

Contents

1. Introduction

The incidence of obesity, a major risk factor for numerous disorders, including diabetes, hypertension and heart disease, is rising at an alarming rate in much of the world (Flier, 2004). Body weight is generally accepted to be determined by the balance between energy intake and expenditure. Normal weight individuals are reportedly protected against the expansion of body fat stores induced by overfeeding (Leibel et al., 1995), indicating the existence of biological mechanisms which protect against weight gain, as well as weight loss,

* Corresponding author. Tel./fax: +81 22 717 8228.
E-mail address: katagiri@mail.tains.tohoku.ac.jp (H. Katagiri).

0163-7258/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2007.09.006

at least in normal weight individuals. Energy homeostasis, maintained by multiple mechanisms, involves collecting information on systemic nutritional status and responding appropriately, both behaviorally and metabolically, to changes in fuel availability. Humoral factors, including insulin and adipocytokines, are known to be very important for this inter-organ/tissue communication. In addition, we and other investigators have recently demonstrated the autonomic nervous system to have a key role in transmitting metabolic information. Employing these systems, the brain gathers information on peripheral metabolic status, processes it, and then sends signals which regulate metabolism in the periphery. The hypothalamus, in particular, is a primary site of convergence and integration for redundant energy status signaling, which encompasses both central and peripheral neural inputs as well as hormonal and nutritional factors. These inter-tissue communication pathways are summarized in (Fig. 1; Yamada & Katagiri, 2007).

All but the most severe obesity cases can be successfully managed, solely through lifestyle modifications, i.e., improvements in diet and promotion of greater physical activity. However, low compliance with these strategies has generated interest in alternative effective therapies, including gastrointestinal bypass surgery (efficacious and long-lasting, but limited in use because of associated risks and costs) and pharmacological interventions. The market for safe and efficacious drugs is therefore potentially enormous, though the value of currently approved therapies does not reflect this potential, due to the limited efficacies and side-effect profiles of these treatments. This review summarizes our current understanding of the roles of inter-tissue communication in energy homeostasis and suggests potential therapeutic targets for obesity and the metabolic syndrome.

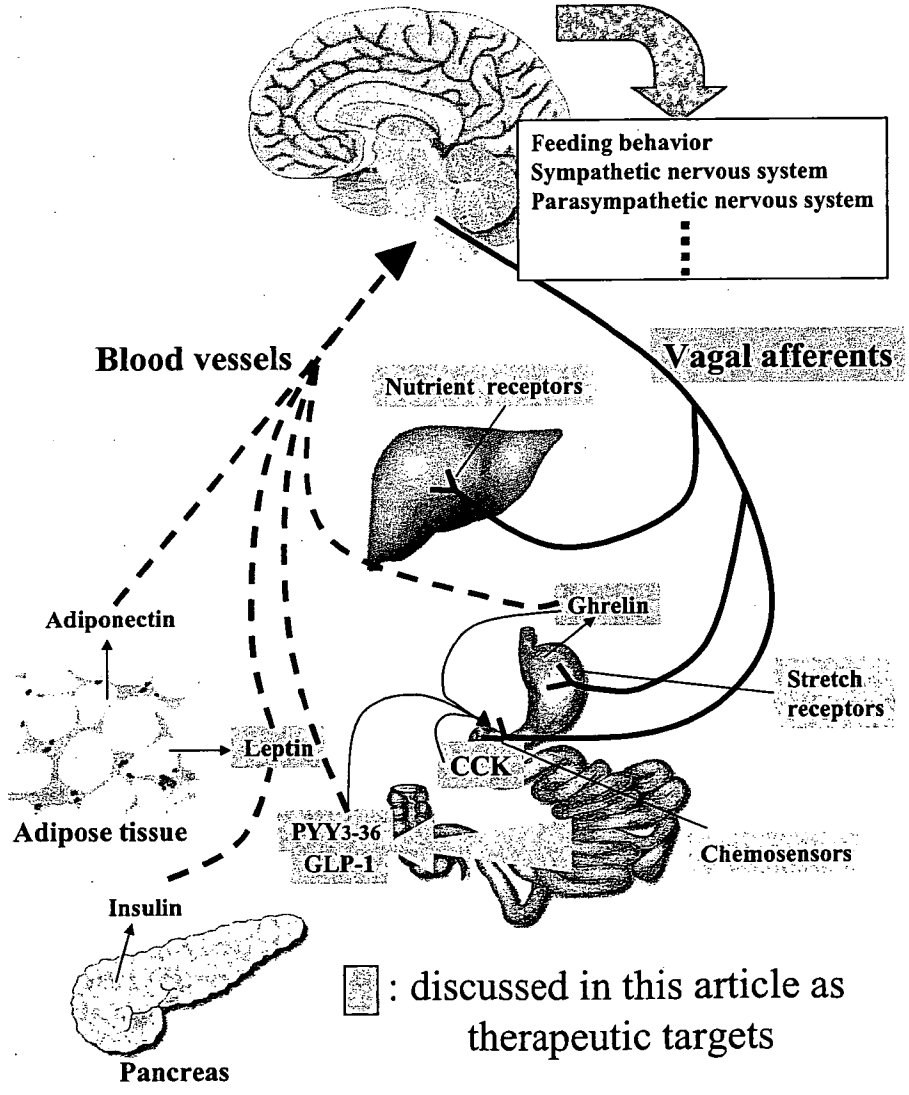


Fig. 1. Schematic presentation of intertissue communication (quoted with slight modification from Yamada & Katagiri, 2007). The brain receives various forms of metabolic information from peripheral organs/tissues through humoral and neuronal pathways. These inputs are probably integrated and processed in the brain, leading to appropriate systemic responses. Several signals, as therapeutic targets, are discussed in this article.

2. Neuroendocrine regulation of body weight and therapeutic implications for obesity

2.1. Brain inputs — humoral factors

2.1.1. Nutrients

The brain senses and then responds to nutrient-related signals arising from changes in intracellular energy contents or in either the availability or metabolism of substrates, such as free fatty acids. Some of these signals are generated in response to decreases in substrates, while others represent responses to nutrient excesses.

2.1.1.1. Glucose. In addition to serving as the primary fuel source for the brain, glucose metabolism in a subset of neurons (so-called "glucose-responsive" and "glucose-sensitive" neurons) generates signals that regulate membrane potential and neuronal firing. In glucose-responsive neurons, the molecular mechanism underlying this glucose effect resembles that, whereby glucose stimulates insulin secretion from pancreatic β cells, resulting in increased firing rates (Rowe et al., 1996). Such neurons have been characterized mainly in the ventromedial hypothalamic (VMH) nucleus and the arcuate (ARC) nucleus (Levin et al., 2004). Glucose metabolism in these cells activates KATP channels, allowing K^+ efflux, and thereby hyperpolarize the cells. KATP channel activity is a key step in converting metabolic changes into the electrical activity of ARC and VMH neurons (Wang et al., 2004). Interestingly, KATP channel activation by glucose in ARC glucose-responsive neurons is attenuated by insulin and leptin via a phosphatidylinositol 3OH kinase (PI3K)-dependent mechanism (Spanswick et al., 2000). Neither the underlying mechanism nor the extent to which these glucose-sensing neurons contribute to the actions of insulin and leptin, in neuroendocrine control of energy homeostasis, has as yet been determined.

In contrast, in glucose-sensitive neurons, firing is suppressed by glucose (Levin et al., 1999). Membrane potential effects mediated by tandem-pore K^+ (K_{2P}) channels were recently reported to be involved in glucose-induced inhibition of orexin neurons, a subset of glucose-sensitive neurons (Burdakov et al., 2006). In these neurons (in this case), glucose metabolism to ATP is not required. Thus, several types of potassium channels, including KATP and K_{2P} channels, are likely to play important roles in glucose sensing in a variety of neurons. However, the molecular mechanisms by which glucose suppresses firing in glucose-sensitive neurons is still largely unknown.

2.1.1.2. Free fatty acids. The access of circulating free fatty acids to cerebrospinal fluids is generally proportional to the plasma fatty acid concentration (Miller et al., 1987; Rapoport, 1996), indicating the brain to possibly acquire information about the peripheral metabolic state via cerebrospinal fluid fatty acid levels. Fatty acid-sensitive neurons have been identified in the hypothalamus. As an example, an *in vitro* patch clamp study (Wang et al., 2006) demonstrated 13% of arcuate neurons to show increased electrical activity, while 6% showed decreased activity, when oleic acid was applied. Several recent studies have examined the role of cerebrospinal fluid fatty acids in energy metabolism. Intracerebroventricular (i.c.v.) administration of oleic acid reportedly inhibits both hepatic glucose production and food intake (Obici et al., 2002). In addition, hypothalamic inhibition of carnitine/palmitoyl-coenzyme A transferase-1 (CPT-1), an important mitochondrial enzyme for transfer of long-chain fatty acyl-coenzyme A (LCFA-CoA) into mitochondria, decreases food intake and suppresses endogenous glucose production (EGP) in the liver (Obici et al., 2003). Efferent vagal nerve signals from the brain to the liver are also reportedly involved in hepatic gluconeogenesis in these experimental settings (Pocai et al., 2005a, 2005b). Hu et al. found that central administration of C75, a potent fatty acid synthase (FAS) inhibitor, decreased food intake (Hu et al., 2003). Since FAS inhibition increases malonyl-CoA and thereby suppresses CPT1 activity, LCFA-CoA in hypothalamic neurons would appear to be increased. These results, taken together, indicate the cytoplasmic LCFA-CoA concentration in hypothalamic neurons to play an important role in energy homeostasis. Further studies are needed to clarify the mechanisms regulating the neuronal LCFA-CoA content, its relationship to plasma free fatty acid (FFA) levels and the intracellular mechanism whereby a change in the LCFA-CoA content alters neuronal function.

2.1.1.3. Amino acids. Amino acids also apparently transmit energy status information from the periphery. Amino acids are reportedly transported across the blood–brain barrier (Choi et al., 2001). Therefore, the amino acid levels in cerebrospinal fluids appear to reflect those in peripheral blood. Centrally administered leucine increases hypothalamic mammalian target of rapamysin (mTOR) activity, thereby decreasing both food intake and body weight (Cota et al., 2006). mTOR is a highly conserved serine/threonine kinase found in organisms from yeast to mammals. mTOR activity sensitive to branched chain amino acid levels, especially that of L-leucine (Proud, 2002; Meijer & Dubbelhuis, 2004). Thus, mTOR is known to be among the energy sensors for amino acids conserved throughout evolution and, in mammals, hypothalamic mTOR signaling apparently plays an important role in regulating systemic energy metabolism. Leptin increases hypothalamic mTOR activity, and inhibition of mTOR signaling suppresses leptin's anorectic effect (Cota et al., 2006). However, further studies are needed to fully clarify mTOR's role in energy homeostasis.

2.1.2. Hormonal signals

2.1.2.1. Insulin. Insulin, produced by pancreatic β cells, is the master metabolic switch between fed and fasted states, mediating metabolic fuel disposition and use. Some investigators speculate that insulin itself might signal fuel status to the brain, but the actual mechanisms by which insulin would exert such effects have long eluded clarification.

An electrophysiological study showed inhibitors of PI3K to block the capacity of insulin to hyperpolarize hypothalamic "glucose-responsive" neurons (Spanswick et al., 2000). A subsequent in vivo study showed i.c.v. infusion of PI3K inhibitors to effectively prevent insulin-induced anorexia (Niswender et al., 2003). Furthermore, activation of insulin signaling in the ARC

alone, in the absence of elevated systemic insulin, is sufficient to decrease not only food intake but also blood glucose levels, by markedly inhibiting EGP in the liver (Plum et al., 2006; Prodi & Obici, 2006). A recent study revealed the central effects of insulin on this hepatic EGP suppression to be mediated by KATP channel activation through the insulin receptor (IR)–insulin receptor substrate 2 (IRS2)–PI3K pathway in the ARC (Pocai et al., 2005a, 2005b). Thus, intracellular insulin signal transduction in the brain, particularly in the hypothalamic ARC nucleus, plays an important role in regulating food intake, as well as in systemic glucose metabolism.

2.1.2.2. Leptin. Leptin was identified by positional cloning using the *ob/ob* mouse model (Zhang et al., 1994) as a key molecule in the regulation of both body weight and energy balance. Leptin is produced mainly by adipocytes in proportion to fat stores. Adipocyte leptin expression is transcriptionally regulated, being determined mainly by adipocyte size. Adequate leptin levels communicate the status of energy stores in white adipose tissue (WAT) to the central nervous system (especially the hypothalamus), suppressing food intake and permitting energy expenditure via sympathetic stimulation of several tissues (Haynes et al., 1997; Friedman & Halaas, 1998). As an example, when energy stores increase, the energy balance is negatively regulated by decreased food intake and increased energy expenditure (Friedman & Halaas, 1998). Leptin binds to the leptin receptor Ob-Rb in the hypothalamus, thereby activating the JAK-STAT (Bjorbaek et al., 1997; Bates & Myers, 2004) and IRS2-PI3K (Niswender et al., 2001) pathways. Leptin also suppresses hypothalamic AMPK activity and thus reduces food intake (Minokoshi et al., 2004). As described above, leptin also activates mTOR signaling in the hypothalamus. Thus, there appear to be complicated interactions among the (at least) 4 pathways, JAK-STAT, IRS2-PI3K, AMPK and mTOR, involved in leptin signaling.

In most individuals with ordinary obesity, circulating leptin is elevated, but the body does not adequately respond to higher leptin levels by reducing food intake. This lack of responsiveness to leptin in most forms of obesity raises the possibility that obesity is a state of relative leptin resistance. Leptin resistance is thought to be an important mechanism for maintaining the obese state.

2.2. Brain inputs — afferent nerve signals

2.2.1. Innervation

2.2.1.1. Intra-abdominal innervation without white adipose tissues. Innervation of intra-abdominal tissues warrants an explanation. Intra-abdominal tissues are innervated by both splanchnic (sympathetic) and vagal (parasympathetic) nerves. These nerve bundles consist of both efferent and afferent fibers. Detailed fiber count studies have revealed abdominal vagal and splanchnic nerves to be comprised of approximately 75% and 50% afferent fibers, respectively. Vagal afferents respond to specific chemical stimuli, the degree of physiological gut distention and nutrients, whereas splanchnic afferents carry information about noxious stimuli (Badman & Flier, 2005).

2.2.1.2. Innervation of intra-abdominal adipose tissues. WAT is also innervated by both efferent and afferent nerve fibers. Numerous reports have described the important metabolic roles, including lipolysis and β oxidation (Shimazu, 1981; Bartness & Bamshad, 1998; Imai et al., 2006), of efferent sympathetic fibers. Efferent parasympathetic innervation of WAT is controversial (Kreier et al., 2002; Giordano et al., 2006). On the other hand, afferent nerves from WAT have been demonstrated by several methods. Sensory innervation of WAT was directly demonstrated using a neuroanatomical approach with application of an anterograde tract tracer, True Blue, to WAT, resulting in labeling of neurons in rat dorsal root ganglia (Fishman & Dark, 1987). More recently, afferent innervation of epididymal WAT was demonstrated by another group using the pseudorabies virus as a retrograde neuronal tracer (Kreier et al., 2006).

2.2.2. Signals transmitted by afferent autonomic nerve fibers

2.2.2.1. Signals from the gut. Many peptides are synthesized and released by the gastrointestinal tract. Several of these peptides, such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and ghrelin, have been shown to modulate eating behaviors, (Woods & Gibbs, 1989; Stanley et al., 2004; Woods, 2004). Several of these peptides have direct access to brain regions involved in regulating food intake, such as the ARC of the hypothalamus and the area postrema, via the circulation. These peptides also function outside the CNS, influencing the activities of neurons, e.g., the vagal afferent nerve which projects to the nucleus of the solitary tract (NTS) in the brain stem. Further research is needed to determine the weighting and integration of each of these different signals.

2.2.2.1.1. CCK. CCK, produced by mucosal enteroendocrine cells of the duodenum and jejunum, is secreted in response to the presence of food in the gut lumen. Satiating effects of CCK have been confirmed based on the carboxy-terminal octapeptide of CCK reducing meal size and duration (Pi-Sunyer et al., 1982). Pharmacologic and genetic experiments have yielded evidence that the CCK1 receptor mediates CCK-induced satiation (Moran et al., 1998; Kopin et al., 1999). Sulfated CCK, which preferentially binds to CCK1R on vagal afferent neurons, signals satiety to the brain; this explains why vagotomy inhibits the anorectic effect of CCK (Smith et al., 1981). However, CCK1R is also expressed in both the hindbrain and the hypothalamus. Lesioning the hindbrain area postrema attenuates CCK-induced satiation (Edwards et al., 1986) and CCK microinjections into several hypothalamic nuclei lead to decreased food intakes (Blevins et al., 2000). Collectively, these observations suggest that CCK might relay satiation signals to the brain both directly and indirectly.

Continuously administering intraperitoneal CCK to rats results in reduced meal sizes, but this reduction is offset by increased meal frequency, such that there is no effect on body weight (West et al., 1984). In human subjects, food intake and gastric emptying were acutely reduced by CCK infusions (Muurahainen et al., 1988), but these anorectic effects disappeared after only 24 hr of continuous infusion (Crawley & Beinfeld, 1983). Therefore, although CCK clearly plays a role in terminating individual

meals, it appears to have little impact on long-term body weight regulation and thus seems unlikely to be an antiobesity drug target.

2.2.2.1.2. PYY3-36. The secretion of PYY3-36 from enteroendocrine L cells is triggered by luminal nutrients. Sugars activate L cells via the closure of ATP-sensitive potassium channels, which in turn leads to depolarization of the L cells via a mechanism analogous to insulin secretion from β cells (Reimann & Gribble, 2002; Gribble et al., 2003). PYY1-36 binds to all known Y receptors with similar affinities. In contrast, most circulating PYY immunoreactivity is in the amino-terminally truncated form, PYY3-36, which preferentially binds to Y2 receptors (Y2R). In the hypothalamus, Y2R is a presynaptic autoinhibitory receptor on orexigenic neurons expressing both NPY and agouti-related protein (AgRP), known as NPY/AgRP neurons. This has lead to the proposal that circulating PYY3-36 reduces food intake by inhibiting NPY/AgRP neurons through Y2R, thereby activating anorectic melanocortin-producing cells, which are inhibited by NPY/AgRP neurons (Batterham et al., 2002). Consistent with this model, the anorectic effects of PYY3-36 can be abolished by either pharmacologic or genetic ablation of Y2R (Batterham et al., 2002; Scott et al., 2005; Talsania et al., 2005). Though these lines of evidence support a hypothalamic mechanism of action for peripherally administered PYY3-36, Y2R is also expressed by vagal-afferent terminals (Koda et al., 2005), and some investigators have speculated that vagal mediation also exists. Supporting this hypothesis, the anorectic effects and arcuate neuronal activation induced by peripheral PYY3-36 were demonstrated to be eliminated by either subdiaphragmatic vagotomy or transection of hindbrain-hypothalamic pathways (Abbott et al., 2005; Koda et al., 2005). Assessed collectively, these observations suggest that PYY3-36 might also relay satiation signals, both direct and indirect, to the brain.

Therapeutic possibilities. Peripheral administration of PYY3-36 reduces food intake, and thereby body weight, in rodents, and these effects may be even more robust in primates (Moran et al., 2005), apparently due to activation of autoinhibitory Y2R on hypothalamic orexigenic NPY/AgRP neurons. In addition, recent evidence indicates vagal mediation of one component of PYY3-36-induced anorexia as described above. Intravenous infusion of a single dose (Batterham et al., 2003), as well as graded infusions (Degen et al., 2005) of PYY3-36, reportedly reduce appetite and food consumption by >30% in lean and obese subjects, although several investigators have encountered difficulties in attempting to reproduce these effects (Boggiano et al., 2005). It is noteworthy that obese subjects show normal sensitivity to the anorectic effects of PYY3-36, and circulating PYY levels are not elevated in the obese, in contrast to those of leptin (Batterham et al., 2003). The injectable PYY3-36 analogue AC-162352 was tested in phase I studies, with limited success due to nausea (Halford, 2006). In a phase I clinical trial as a nasally administered obesity treatment, on the other hand, PYY3-36 was both safe and well tolerated, and there was evidence of reduced caloric intake, appetite moderation and a tendency for weight loss in human subjects (Halford, 2006).

2.2.2.1.3. Glucagon-like peptide-1. GLP-1 is produced mainly by L cells located in the distal small intestine and colon, where it is colocalized with PYY. Ingested nutrients, especially fats and carbohydrates, stimulate GLP-1 secretion by indirect, duodenally activated neurohumoral mechanisms, as well as via direct contact within the distal intestine (Brubaker & Anini, 2003). The 2 equipotent bioactive forms, GLP-17-36 amide and GLP-17-37, are rapidly inactivated in the circulation by dipeptidyl peptidase-IV (DPP-IV; Orskov et al., 1993). GLP-1 has been shown to suppress food intake in several species (Turton et al., 1996; Donahey et al., 1998), including humans (Verdich et al., 2001). The mechanisms underlying GLP-1-induced anorexia are not fully understood but are believed to involve vagal and possibly direct central pathways. The GLP-1 receptor is expressed by organs/tissues, including the gut, pancreas, brainstem, hypothalamus and vagal-afferent nerves (Drucker, 2006). The anorectic effects of peripheral GLP-1 administration were shown to be abolished by vagotomy (Abbott et al., 2005; Talsania et al., 2005). Thus, peripheral GLP-1 also signals satiety to the brain via the vagal afferent pathway.

Therapeutic possibilities. Chronic subcutaneous GLP-1 administration for 6 weeks to obese subjects with type 2 diabetes led to a 1.9-kg body weight loss, on average (Zander et al., 2002). Given that the native GLP-1 peptide undergoes rapid enzymatic inactivation, DPP-IV-resistant GLP-1 analogues have attracted considerable attention as potential treatments for type 2 diabetes complicated by obesity. Exendin-4 is a naturally occurring 39 amino acid GLP-1 receptor agonist originally isolated from the venom of the *Heloderma suspectum* lizard (Eng et al., 1992). Exendin-4 has a glycine residue, which confers resistance to cleavage by DPP-IV, at position 2. In clinical trials, twice-daily subcutaneous administration of exendin-4 in patients with type 2 diabetes produced a dose-dependent weight loss of 1.8 kg during a period of 28 days (Poon et al., 2005) and 2.8 kg over 30 weeks (DeFronzo et al., 2005). Thus, in addition to promoting insulin secretion (incretin effects), the anorectic effects of GLP-1 agonists have attracted attention as possible diabetes treatments. This is because the improvements in glycemic control achieved with other oral glucose-lowering drugs typically promote weight gain.

2.2.2.1.4. Ghrelin. Ghrelin, a peptide recently found to be produced by the stomach, acts on a previously identified orphan receptor (growth hormone [GH] secretagogue receptor), the activation of which in the hypothalamus triggers the pituitary gland to release GH (Kojima et al., 1999). Ghrelin increases food intake in diverse species (Tschop et al., 2000), including humans (Wren et al., 2001). Date et al. (2002) reported gastric vagal afferent blockade to abolish ghrelin-induced feeding increases, GH secretion and the activations of NPY- and GH-releasing hormone (GHRH)-producing neurons. The ghrelin receptor is also expressed in vagal afferent terminals, and ghrelin suppresses vagal afferent firing. These findings, taken together, indicate gastric vagal afferent involvement in conveying signals regarding starvation, as well from the gut to the brain.

Therapeutic possibilities. In humans, feelings of hunger and food intake are both increased by either intravenous infusion or subcutaneous injection of ghrelin (Kojima & Kangawa, 2005). Therefore, blocking ghrelin signaling with ghrelin receptor antagonists has attracted interest as a possible strategy for preventing obesity. A ghrelin receptor antagonist reportedly

reduced food intake in fasted mice and an RNA Spiegelmer (an L-oligonucleotide designed to bind specifically to a particular molecule) inhibited ghrelin action both in vitro and in vivo (Cummings, 2006). It was recently demonstrated that vaccinating rats against ghrelin can suppress weight gain (Zorrilla et al., 2006). However, in obese individuals,ghrelin levels are low but rise in response to weight loss (Kojima & Kangawa, 2005). This is apparently part of a compensatory response that promotes weight regain. Therefore, the most clinically useful application of ghrelin receptor blockade might be in the prevention of rebound after weight reduction, which has been achieved by other means, rather than for initiating weight reduction de novo.

2.2.2.2. Signals from the liver

2.2.2.2.1. Hepatoportal glucose sensor. Sensor of short-term alterations in energy status. Blood glucose levels rise postprandially and decrease while fasting. Therefore, blood glucose concentrations reflect short-term energy status alterations. Glucose absorbed from the gut enters the portal vein, thereby reaching the liver directly. Thus, given its anatomical location, it is reasonable to assume that the liver functions as a glucose sensor. The hepatoportal glucose sensor is as yet incompletely defined, but reportedly consists of several components including GLUT2 (Burcelin et al., 2000a), as well as the GLP-1 receptor (Burcelin et al., 2001). Glucose entry into the hepatoportal vein triggers the activation of glucose sensors (Hevener et al., 1997), which can induce anorexia (Russek, 1963, 1970) and stimulate glucose uptake by the liver (Gardemann et al., 1986; Cardin et al., 1999), muscle, heart and brown adipose tissue (Burcelin et al., 2000b). Raising the portal vein glucose concentration decreases vagal afferent discharges reaching the NTS nuclei (Thorens & Larsen, 2004), indicating signals regarding portal glucose elevation to be carried along afferent vagal pathways. Hypoglycemic signals from the hepatoportal system, in contrast, involve splanchnic afferents. Reportedly, a counter-regulatory response to moderate systemic hypoglycemia, i.e., sympathetic efferent activation, is attenuated by clamping the liver at euglycemic levels and is blocked when splanchnic (but not vagal) afferents from the hepatic portal structure are interrupted (Donovan et al., 1991; Fujita & Donovan, 2005).

These observations, when considered collectively, indicate that the afferent autonomic nervous system, including both vagal and splanchnic nerves, from the hepatoportal circulation plays important roles in conveying information about peripheral glucose levels to the brain.

2.2.2.2.2. Peroxisome proliferator-activated receptors. Sensor of long-term alterations in energy status. Lipid mediators have key roles in metabolic control, and the peroxisome proliferator-activated receptor (PPAR) have emerged as the master transcriptional regulators of long-term lipid and carbohydrate metabolism (Desvergne et al., 2006). Saturated and unsaturated long-chain fatty acids and their eicosanoid derivatives are natural activators of this important subclass of nuclear receptors (Feige et al., 2006). Studies using mice with tissue-specific knockout of PPARγ have shown these receptors, in a number of organs, to function as a sensor of long-term energy status alterations. Notably, liver-specific disruption of PPARγ in ob/ob mice prevented hepatic steatosis, although a gradual increase in peripheral adiposity as well as decreases in the insulin sensitivities of muscle and adipose tissue were observed (Matsusue et al., 2003). In addition, hepatic expression of PPARγ, especially that of PPARγ2, is functionally enhanced in a number of obesity models (Chao et al., 2000; Rahimian et al., 2001). Therefore, hepatic PPARγ appears to play important roles not only in hepatic lipid storage but also in the regulation of both peripheral lipid metabolism and insulin sensitivity. The mechanism underlying this inter-organ/tissue communication between the liver and peripheral tissues, including muscle and fat, was recently revealed to involve autonomic nerve circuits (Uno et al., 2006).

The roles of hepatic PPARγ2 in peripheral metabolism were confirmed experimentally. Adenovirus-mediated PPARγ2 expression in the liver was shown to acutely induce severe hepatic steatosis, while peripheral adiposity was greatly reduced due to enhanced lipolysis. Systemic metabolic rates rose, such peripheral insulin sensitivity, and glucose tolerance showed marked improvements. These remote effects were attributable to increased sympathetic outflow into muscle and adipose tissues. Selective hepatic branch vagotomy significantly reversed both the peripheral adiposity reduction and the enhanced energy expenditure. Furthermore, pharmacological deafferentation of the vagus blocked the hepatic PPARγ2 expression-induced decrease in WAT weights. These findings indicate that hepatic PPARγ2 expression and/or hepatic lipid accumulation triggers the communication of metabolic information to the brain via afferent vagal nerve fibers, leading to antiobesity and antiinsulin-resistant effects in both muscle and adipose tissue (Uno et al., 2006).

Lipid storage in the liver changes dynamically according to the systemic energy balance and is known to be associated with several features of the metabolic syndrome. The liver may convey information regarding excess long-term energy storage to the central nervous system via the afferent vagus. This neuronal system is likely to underlie so-called chronic "adaptive thermogenesis," protecting the organism against metabolic perturbation induced by excessive energy storage (Fig. 2). The brain receives information regarding this excess energy storage, via leptin from adipose tissues as well as via the afferent vagus from the liver, activates the sympathetic nervous system to enhance energy expenditure and lipolysis, and thereby maintains energy homeostasis (Uno et al., 2006). A similar autonomic nerve circuit was recently shown to play an essential role in the development of glucocorticoid-induced insulin resistance and hypertension (Bernal-Mizrachi et al., 2007).

In totality, these observations highlight the importance of the vagal afferent pathway not only in short-term nutrient status alterations, such as blood glucose concentrations, but also in long-term energy storage status alterations.

Therapeutic possibilities. A recent study found a low resting metabolic rate to predict susceptibility to obesity (Buscemi et al., 2005). Therefore, enhancing energy expenditure is a promising strategy for treating obesity. Physiological sympathetic activation might thus be feasible, because it leads to relatively selective loss of fat, followed by improvements in insulin sensitivity beyond what would be expected from body weight reduction.

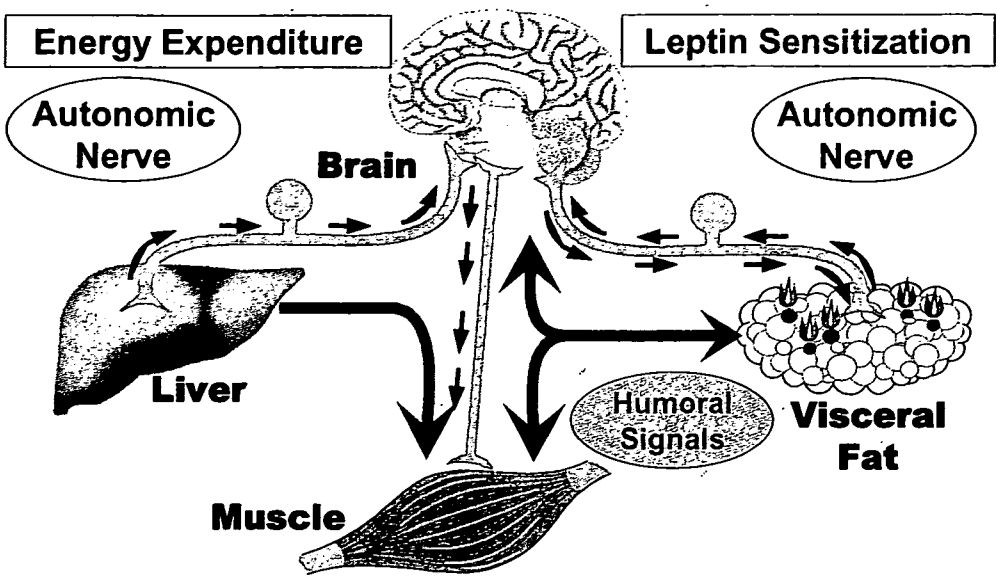


Fig. 2. Scheme of the neuronal pathways involved in energy homeostasis. Neuronal signals from WAT modulate hypothalamic leptin sensitivity, thereby regulating feeding behavior. In addition, the liver transmits information regarding excess energy to the brain via the afferent vagus, thereby activating the sympathetic nervous system which in turn enhances energy expenditure and lipolysis. The autonomic nervous system, as exemplified by these neuronal circuits, plays important roles in regulating energy metabolism.

Sibutramine, a serotonin-norepinephrine reuptake inhibitor, which is used clinically as an antiobesity drug, apparently increases sympathetic activity, though whether this would contribute in any way to weight loss in humans is unclear (Luque & Rey, 2002). As noted above, the autonomic nerve circuit consisting of the afferent vagus nerve and efferent sympathetic nervous system has a physiological antiobesity function exerted through enhanced energy expenditure. Therefore, in addition to reagents acting directly on the brain, activators of this peripheral nervous system are potential antiobesity drug targets. Elucidation of the underlying molecular mechanisms, including mediators influencing vagal activity, could lead to new therapeutic approaches to obesity and the metabolic syndrome. However, sibutramine raises both heart rate and blood pressure. Caution is necessary when any novel agents raises energy expenditure, because these effects may simply be due to an overall increase in systemic sympathetic activity.

The sympathetic nervous system reportedly activates uncoupling protein-1 (UCP-1) expression and activity. UCP-1 is most abundantly expressed in brown adipose tissue and dissipates energy as heat. In addition, transgenic overexpression of UCP-1 in WAT has been reported to exert preventive effects against the development of both genetic and dietary obesity, as well as the associated insulin resistance, in mice (Kopecky et al., 1995, 1996). Furthermore, hepatic induction of UCP-1 protein in mice with dietary obesity improves both diabetes and obesity by exerting local effects in the liver as well as remote effects in adipose tissues, muscle and the hypothalamus (Ishigaki et al., 2005). In contrast, in lean mice fed a standard diet, hepatic UCP-1 expression had little impact on either glucose or lipid metabolism and no cachectic phenotypes were observed (Ishigaki et al., 2005). These observations suggest UCP-1 to possibly be an attractive therapeutic target for both obesity and the metabolic syndrome.

2.2.2.3. Signals from adipose tissues. Few reports have focused on afferent nerve signals from adipose tissues. Niijima (1998) and Tanida et al. (2000) used electrical firing measurements to demonstrate that leptin induces functional activation of afferent nerve fibers from epididymal WAT. The functional roles of these afferent nerves in food intake regulation has been recently shown (Yamada et al., 2006).

Fat accumulation in intra-abdominal adipose tissue plays a major role in development of the metabolic syndrome, which is associated with insulin as well as leptin resistance. As described above, leptin resistance is induced by excessive adiposity and, in turn, is an important mechanism for maintaining the obese state. UCP-1 induction in restricted portions of epididymal adipose tissue, even at very low levels, dramatically improves hypothalamic leptin resistance without altering adiposity and thereby decreases food intake. Locally dissecting nerves from the epididymal fat pad and pharmacological deafferentation blunted these anorectic effects of UCP-1 expression in adipose tissue. Thus, afferent nerve signals originating in epididymal fat pads were shown to modulate hypothalamic sensitivity to leptin (Yamada et al., 2006; Fig. 2). In addition, the involvement of afferent nerves from WAT in adiposity was suggested by the observation that localized selective sensory denervation, achieved by microinjecting capsaicin bilaterally into epididymal WAT of Siberian hamsters, produced increases in other intraabdominal fat masses (Shi & Bartness, 2005).

Adipose tissues were long regarded as simply being passive fuel storage sites. However, the discovery of various adipocytokines, with leptin being the most important example, has raised adipose tissue to the status of a versatile endocrine gland. In addition, these aforementioned studies provide further evidence that adipose tissue serves as a base, sending out neuronal signals regulating feeding and energy storage.

Therapeutic possibilities. From the therapeutic perspective, the mechanism underlying leptin resistance is an important issue awaiting clarification, though two hypotheses have received considerable attention. One involves a failure of circulating leptin to arrive at its targets in the brain. Leptin is normally transported across the blood brain barrier by a saturable transport system, and the activity of this system has been shown to be impaired in obese subjects (Schwartz et al., 1996). Intranasal delivery of leptin can reportedly overcome this barrier and thereby produce weight loss in rats (Fliedner et al., 2006).

Another important observation is that, independently of blood–brain transit, intracellular leptin-receptor signaling is blunted in brain areas critical to energy homeostasis in the setting of diet-induced obesity, such that neuronal leptin responsiveness is diminished even when leptin is directly injected into the brain (El-Haschimi et al., 2000). In fact, several studies support potential roles of two molecules, suppressor of cytokine signaling-3 (SOCS3; Bjorbaek et al., 1998) and protein tyrosine phosphatase-1B (PTP1B; Cheng et al., 2002; Zabolotny et al., 2002), in the inhibitory regulation of Ob-Rb signaling both in vitro and in vivo. Although hypothalamic PTP1B levels do not appear to be altered in obesity, SOCS3 expression is increased in several rodent models of leptin-resistant obesity, which is consistent with the potential role of SOCS3 in leptin resistance (Bjorbaek et al., 1998; Munzberg et al., 2004). Moreover, ablation of SOCS3 activity by employing neuron-specific conditional knockout increases leptin-induced activation of intracellular signaling events and catabolic neuropeptide expressions, associated with enhancement of the weight-reducing effects of leptin and resistance to diet-induced obesity (Howard et al., 2004; Mori et al., 2004). PTP1B also inactivates the leptin receptor via dephosphorylation of its key tyrosine residues, which are phosphorylated in response to ligand binding (Cheng et al., 2002; Zabolotny et al., 2002). Global and neuron-specific PTP1B knockout mice are lean, resistant to diet-induced obesity, and insulin sensitive, all of which result more from increased energy expenditure than decreased food intake (Elchebly et al., 1999; Klaman et al., 2000; Bence et al., 2006). Therefore, SOCS3 and PTP1B are potential therapeutic targets for leptin resistance. Caution is warranted, however, since SOCS3 and PTP1B regulate more than just leptin signaling.

As stated above, neuronal signals from intraabdominal adipose tissue modulate hypothalamic leptin sensitivity (Yamada et al., 2006). Activation of this novel neuronal pathway is a possible therapeutic strategy against obesity and the metabolic syndrome. Elucidating the molecular mechanism(s) underlying this pathway, including identification of the neurotransmitters involved and their receptors, might lead to the development of novel therapeutic strategies, tackling the metabolic syndrome via improved leptin resistance.

3. Epilogue

Metabolism is not a process carried on independently in different organs/tissues, but rather is coordinated and regulated throughout the body. The coordination of metabolic regulation among organs/tissues, which requires communication among these organs/tissues, is apparently essential for maintaining the homeostasis of systemic metabolism, especially glucose and energy metabolism. In addition, disturbances of this coordinated control system may be involved in the development of metabolic disorders, including obesity, type 2 diabetes, hyperlipidemia, and the metabolic syndrome.

Recent research advances have revealed the complex and important roles played by the central nervous system. The brain obtains an abundance of metabolic information from peripheral organs/tissues through humoral and neuronal avenues. In addition, these signals interact, as exemplified by adiponectin expressions being regulated by sympathetic activity (Imai et al., 2006). These inputs are most likely integrated and processed in the brain, leading to the transmission of regulatory signals, which then induce appropriate systemic metabolic responses (Katagiri et al., 2007). Elucidation of these regulatory systems, in far greater detail, may reveal the mechanisms underlying metabolic homeostasis and thereby allow us to understand the complex metabolic disorders that result from perturbation of these systems.

Although life-style change is widely accepted as the first-line treatment for obesity and the metabolic syndrome, in the actual clinical setting, the multiple risks associated with obesity do not normalize with efforts aimed at life-style changes alone. Unfortunately, the existing pharmacological treatments for obesity, which might be used to ameliorate the risks associated with obesity, provide limited efficacy. This lack of efficacy is often further compounded by unacceptable side-effects. Concern about the safety of centrally acting drugs is one reason that pharmaceutical companies are currently seeking alternative obesity treatments. Targeting inter-tissue/organ communication in energy homeostasis might offer advantages in exploiting natural regulatory circuits while minimizing unwanted side effects. Diet and exercise remain the undisputed cornerstones of obesity therapy. However, more effective medications, designed to augment the impacts of these efforts, would be welcomed by obese individuals.

Acknowledgments

This work was supported by grants-in-aid for scientific research (B2, 18390267 to H. Katagiri and B2, 17390258 to Y. Oka) from the Ministry of Education, Science, Sports and Culture of Japan and a grant-in-aid for scientific research (H19 genome-005) from the Ministry of Health, Labor and Welfare of Japan (to Y. Oka). This work was also supported by the 21st Century COE Programs (to H. Katagiri and Y. Oka) by the Ministry of Education Science, Sports and Culture.

References

Abbott, C. R., Monteiro, M., Small, C. J., Sajedi, A., Smith, K. L., Parkinson, J. R., et al. (2005). The inhibitory effects of peripheral administration of peptide YY (3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. *Brain Res 1044* (1), 127–131.

Badman, M. K., & Flier, J. S. (2005). The gut and energy balance: visceral allies in the obesity wars. *Science 307*(5717), 1909–1914.

Bartness, T. J., & Bamshad, M. (1998). Innervation of mammalian white adipose tissue: implications for the regulation of total body fat. *Am J Physiol 275* (5 Pt 2), R1399–R1411.

Bates, S. H., & Myers, M. G. (2004). The role of leptin->STAT3 signaling in neuroendocrine function: an integrative perspective. *J Mol Med 82*(1), 12–20.

Batterham, R. L., Cohen, M. A., Ellis, S. M., Le Roux, C. W., Withers, D. J., Frost, G. S., et al. (2003). Inhibition of food intake in obese subjects by peptide YY3-36. *N Engl J Med 349*(10), 941–948.

Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H., Cohen, M. A., Dakin, C. L., et al. (2002). Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature 418*(6898), 650–654.

Bence, K. K., Delibegovic, M., Xue, B., Gorgun, C. Z., Hotamisligil, G. S., Neel, B. G., et al. (2006). Neuronal PTP1B regulates body weight, adiposity and leptin action. *Nat Med 12*(8), 917–924.

Bernal-Mizrachi, C., Xiaozhong, L., Yin, L., Knutsen, R. H., Howard, M. J., Arends, J. J., et al. (2007). An afferent vagal nerve pathway links hepatic PPARalpha activation to glucocorticoid-induced insulin resistance and hypertension. *Cell Metab 5*(2), 91–102.

Bjorbaek, C., Elmquist, J. K., Frantz, J. D., Shoelson, S. E., & Flier, J. S. (1998). Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell 1*(4), 619–625.

Bjorbaek, C., Uotani, S., da Silva, B., & Flier, J. S. (1997). Divergent signaling capacities of the long and short isoforms of the leptin receptor. *J Biol Chem 272*(51), 32686–32695.

Blevins, J. E., Stanley, B. G., & Reidelberger, R. D. (2000). Brain regions where cholecystokinin suppresses feeding in rats. *Brain Res 860*(1-2), 1–10.

Boggiano, M. M., Chandler, P. C., Oswald, K. D., Rodgers, R. J., Blundell, J. E., Ishii, Y., et al. (2005). PYY3-36 as an anti-obesity drug target. *Obes Rev 6*(4), 307–322.

Brubaker, P. L., & Anini, Y. (2003). Direct and indirect mechanisms regulating secretion of glucagon-like peptide-1 and glucagon-like peptide-2. *Can J Physiol Pharmacol 81*(11), 1005–1012.

Burcelin, R., Dolci, W., & Thorens, B. (2000). Glucose sensing by the hepatoportal sensor is GLUT2-dependent: in vivo analysis in GLUT2-null mice. *Diabetes 49*(10), 1643–1648.

Burcelin, R., Dolci, W., & Thorens, B. (2000). Portal glucose infusion in the mouse induces hypoglycemia: evidence that the hepatoportal glucose sensor stimulates glucose utilization. *Diabetes 49*(10), 1635–1642.

Burcelin, R., Da Costa, A., Drucker, D., & Thorens, B. (2001). Glucose competence of the hepatoportal vein sensor requires the presence of an activated glucagon-like peptide-1 receptor. *Diabetes 50*(8), 1720–1728.

Burdakov, D., Jensen, L. T., Alexopoulos, H., Williams, R. H., Fearon, I. M., O'Kelly, I., et al. (2006). Tandem-pore K+channels mediate inhibition of orexin neurons by glucose. *Neuron 50*(5), 711–722.

Buscemi, S., Verga, S., Caimi, G., & Cerasola, G. (2005). Low relative resting metabolic rate and body weight gain in adult Caucasian Italians. *Int J Obes (Lond) 29*(3), 287–291.

Cardin, S., Emshwiller, M., Jackson, P. A., Snead, W. L., Hastings, J., Edgerton, D. S., et al. (1999). Portal glucose infusion increases hepatic glycogen deposition in conscious unrestrained rats. *J Appl Physiol 87*(4), 1470–1475.

Chao, L., Marcus-Samuels, B., Mason, M. M., Moitra, J., Vinson, C., Arioglu, E., et al. (2000). Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. *J Clin Invest 106*(10), 1221–1228.

Cheng, A., Uetani, N., Simoncic, P. D., Chaubey, V. P., Lee-Loy, A., McGlade, C. J., et al. (2002). Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. *Dev Cell 2*(4), 497–503.

Choi, Y. H., Fletcher, P. J., & Anderson, G. H. (2001). Extracellular amino acid profiles in the paraventricular nucleus of the rat hypothalamus are influenced by diet composition. *Brain Res 892*(2), 320–328.

Cota, D., Proulx, K., Smith, K. A., Kozma, S. C., Thomas, G., Woods, S. C., et al. (2006). Hypothalamic mTOR signaling regulates food intake. *Science 312* (5775), 927–930.

Crawley, J. N., & Beinfeld, M. C. (1983). Rapid development of tolerance to the behavioural actions of cholecystokinin. *Nature 302*(5910), 703–706.

Cummings, D. E. (2006). Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiol Behav 89*(1), 71–84.

Date, Y., Murakami, N., Toshinai, K., Matsukura, S., Niijima, A., Matsuo, H., et al. (2002). The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology 123*(4), 1120–1128.

DeFronzo, R. A., Ratner, R. E., Han, J., Kim, D. D., Fineman, M. S., & Baron, A. D. (2005). Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care 28*(5), 1092–1100.

Degen, L., Oesch, S., Casanova, M., Graf, S., Ketterer, S., Drewe, J., et al. (2005). Effect of peptide YY3-36 on food intake in humans. *Gastroenterology 129*(5), 1430–1436.

Desvergne, B., Michalik, L., & Wahli, W. (2006). Transcriptional regulation of metabolism. *Physiol Rev 86*(2), 465–514.

Donahey, J. C., van Dijk, G., Woods, S. C., & Seeley, R. J. (1998). Intraventricular GLP-1 reduces short-but not long-term food intake or body weight in lean and obese rats. *Brain Res 779*(1-2), 75–83.

Donovan, C. M., Halter, J. B., & Bergman, R. N. (1991). Importance of hepatic glucoreceptors in sympathoadrenal response to hypoglycemia. *Diabetes 40*(1), 155–158.

Drucker, D. J. (2006). The biology of incretin hormones. *Cell Metab 3*(3), 153–165.

Edwards, G. L., Ladenheim, E. E., & Ritter, R. C. (1986). Dorsomedial hindbrain participation in cholecystokinin-induced satiety. *Am J Physiol 251*(5 Pt 2), R971–R977.

Elchebly, M., Payette, P., Michaliszyn, E., Cromlish, W., Collins, S., Loy, A. L., et al. (1999). Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science 283*(5407), 1544–1548.

El-Haschimi, K., Pierroz, D. D., Hileman, S. M., Bjorbaek, C., & Flier, J. S. (2000). Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest 105*(12), 1827–1832.

Eng, J., Kleinman, W. A., Singh, L., Singh, G., & Raufman, J. P. (1992). Isolation and characterization of exendin-4, an exendin-3 analogue, from *Heloderma suspectum* venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J Biol Chem 267*(11), 7402–7405.

Feige, J. N., Gelman, L., Michalik, L., Desvergne, B., & Wahli, W. (2006). From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions. *Prog Lipid Res 45*(2), 120–159.

Fishman, R. B., & Dark, J. (1987). Sensory innervation of white adipose tissue. *Am J Physiol 253*(6 Pt 2), R942–R944.

Fliedner, S., Schulz, C., & Lehnert, H. (2006). Brain uptake of intranasally applied radioiodinated leptin in Wistar rats. *Endocrinology 147*(5), 2088–2094.

Flier, J. S. (2004). Obesity wars: molecular progress confronts an expanding epidemic. *Cell 116*(2), 337–350.

Friedman, J. M., & Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature 395*(6704), 763–770.

Fujita, S., & Donovan, C. M. (2005). Celiac-superior mesenteric ganglionectomy, but not vagotomy, suppresses the sympathoadrenal response to insulin-induced hypoglycemia. *Diabetes 54*(11), 3258–3264.

Gardemann, A., Strulik, H., & Jungermann, K. (1986). A portal-arterial glucose concentration gradient as a signal for an insulin-dependent net glucose uptake in perfused rat liver. *FEBS Lett 202*(2), 255–259.

Giordano, A., Song, C. K., Bowers, R. R., Ehlen, J. C., Frontini, A., Cinti, S., et al. (2006). White adipose tissue lacks significant vagal innervation and immunohistochemical evidence of parasympathetic innervation. *Am J Physiol Regul Integr Comp Physiol 291*(5), R1243–R1255.

Gribble, F. M., Williams, L., Simpson, A. K., & Reimann, F. (2003). A novel glucose-sensing mechanism contributing to glucagon-like peptide-1 secretion from the GLUTag cell line. *Diabetes 52*(5), 1147–1154.

Halford, J. C. (2006). Obesity drugs in clinical development. *Curr Opin Investig Drugs 7*(4), 312–318.

Haynes, W. G., Morgan, D. A., Walsh, S. A., Mark, A. L., & Sivitz, W. I. (1997). Receptor-mediated regional sympathetic nerve activation by leptin. *J Clin Invest 100*(2), 270–278.

Hevener, A. L., Bergman, R. N., & Donovan, C. M. (1997). Novel glucosensor for hypoglycemic detection localized to the portal vein. *Diabetes 46*(9), 1521–1525.

Howard, J. K., Cave, B. J., Oksanen, L. J., Tzameli, I., Bjorbaek, C., & Flier, J. S. (2004). Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. *Nat Med 10*(7), 734–738.

Hu, Z., Cha, S. H., Chohnan, S., & Lane, M. D. (2003). Hypothalamic malonyl-CoA as a mediator of feeding behavior. *Proc Natl Acad Sci U S A 100*(22), 12624–12629.

Imai, J., Katagiri, H., Yamada, T., Ishigaki, Y., Ogihara, T., Uno, K., et al. (2006). Cold exposure suppresses serum adiponectin levels through sympathetic nerve activation in mice. *Obesity (Silver Spring) 14*(7), 1132–1141.

Ishigaki, Y., Katagiri, H., Yamada, T., Ogihara, T., Imai, J., Uno, K., et al. (2005). Dissipating excess energy stored in the liver is a potential treatment strategy for diabetes associated with obesity. *Diabetes 54*(2), 322–332.

Katagiri, H., Yamada, T., & Oka, Y. (2007). Adiposity and cardiovascular disorders: disturbance of the regulatory system consisting of humoral and neuronal signals. *Circ Res 101*(1), 27–39.

Klaman, L. D., Boss, O., Peroni, O. D., Kim, J. K., Martino, J. L., Zabolotny, J. M., et al. (2000). Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol Cell Biol 20*(15), 5479–5489.

Koda, S., Date, Y., Murakami, N., Shimbara, T., Hanada, T., Toshinai, K., et al. (2005). The role of the vagal nerve in peripheral PYY3-36-induced feeding reduction in rats. *Endocrinology 146*(5), 2369–2375.

Kojima, M., & Kangawa, K. (2005). Ghrelin: structure and function. *Physiol Rev 85*(2), 495–522.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature 402*(6762), 656–660.

Kopecky, J., Clarke, G., Enerback, S., Spiegelman, B., & Kozak, L. P. (1995). Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. *J Clin Invest 96*(6), 2914–2923.

Kopecky, J., Hodny, Z., Rossmeisl, M., Syrovy, I., & Kozak, L. P. (1996). Reduction of dietary obesity in aP2-Ucp transgenic mice: physiology and adipose tissue distribution. *Am J Physiol 270*(5 Pt 1), E768–E775.

Kopin, A. S., Mathes, W. F., McBride, E. W., Nguyen, M., Al-Haider, W., Schmitz, F., et al. (1999). The cholecystokinin-A receptor mediates inhibition of food intake yet is not essential for the maintenance of body weight. *J Clin Invest 103*(3), 383–391.

Kreier, F., Fliers, E., Voshol, P. J., Van Eden, C. G., Havekes, L. M., Kalsbeek, A., et al. (2002). Selective parasympathetic innervation of subcutaneous and intra-abdominal fat-functional implications. *J Clin Invest 110*(9), 1243–1250.

Kreier, F., Kap, Y. S., Mettenleiter, T. C., van Heijningen, C., van der Vliet, J., Kalsbeek, A., et al. (2006). Tracing from fat tissue, liver, and pancreas: a neuroanatomical framework for the role of the brain in type 2 diabetes. *Endocrinology 147*(3), 1140–1147.

Leibel, R. L., Rosenbaum, M., & Hirsch, J. (1995). Changes in energy expenditure resulting from altered body weight. *N Engl J Med 332*(10), 621–628.

Levin, B. E., Dunn-Meynell, A. A., & Routh, V. H. (1999). Brain glucose sensing and body energy homeostasis: role in obesity and diabetes. *Am J Physiol 276* (5 Pt 2), R1223–R1231.

Levin, B. E., Routh, V. H., Kang, L., Sanders, N. M., & Dunn-Meynell, A. A. (2004). Neuronal glucosensing: what do we know after 50 years? *Diabetes 53*(10), 2521–2528.

Luque, C. A., & Rey, J. A. (2002). The discovery and status of sibutramine as an anti-obesity drug. *Eur J Pharmacol 440*(2-3), 119–128.

Matsusue, K., Haluzik, M., Lambert, G., Yim, S. H., Gavrilova, O., Ward, J. M., et al. (2003). Liver-specific disruption of PPARgamma in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes. *J Clin Invest 111*(5), 737–747.

Meijer, A. J., & Dubbelhuis, P. F. (2004). Amino acid signalling and the integration of metabolism. *Biochem Biophys Res Commun 313*(2), 397–403.

Miller, J. C., Gnaedinger, J. M., & Rapoport, S. I. (1987). Utilization of plasma fatty acid in rat brain: distribution of [14C]palmitate between oxidative and synthetic pathways. *J Neurochem 49*(5), 1507–1514.

Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y. B., Lee, A., Xue, B., et al. (2004). AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature 428*(6982), 569–574.

Moran, T. H., Katz, L. F., Plata-Salaman, C. R., & Schwartz, G. J. (1998). Disordered food intake and obesity in rats lacking cholecystokinin A receptors. *Am J Physiol 274*(3 Pt 2), R618–R625.

Mori, H., Hanada, R., Hanada, T., Aki, D., Mashima, R., Nishinakamura, H., et al. (2004). Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nat Med 10*(7), 739–743.

Moran, T. H., Smedh, U., Kinzig, K. P., Scott, K. A., Knipp, S., & Ladenheim, E. E. (2005). Peptide YY(3-36) inhibits gastric emptying and produces acute reductions in food intake in rhesus monkeys. *Am J Physiol Regul Integr Comp Physiol 288*(2), R384–R388.

Munzberg, H., Flier, J. S., & Bjorbaek, C. (2004). Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology 145*(11), 4880–4889.

Muurahainen, N., Kissileff, H. R., Derogatis, A. J., & Pi-Sunyer, F. X. (1988). Effects of cholecystokinin-octapeptide (CCK-8) on food intake and gastric emptying in man. *Physiol Behav 44*(4-5), 645–649.

Niijima, A. (1998). Afferent signals from leptin sensors in the white adipose tissue of the epididymis, and their reflex effect in the rat. *J Auton Nerv Syst 73*(1), 19–25.

Niswender, K. D., Morrison, C. D., Clegg, D. J., Olson, R., Baskin, D. G., Myers, M. G., Jr., et al. (2003). Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. *Diabetes 52*(2), 227–231.

Niswender, K. D., Morton, G. J., Stearns, W. H., Rhodes, C. J., Myers, M. G., Jr., & Schwartz, M. W. (2001). Intracellular signalling. Key enzyme in leptin-induced anorexia. *Nature 413*(6858), 794–795.

Obici, S., Feng, Z., Arduini, A., Conti, R., & Rossetti, L. (2003). Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production. *Nat Med 9*(6), 756–761.

Obici, S., Feng, Z., Morgan, K., Stein, D., Karkanias, G., & Rossetti, L. (2002). Central administration of oleic acid inhibits glucose production and food intake. *Diabetes 51*(2), 271–275.

Orskov, C., Wettergren, A., & Holst, J. J. (1993). Biological effects and metabolic rates of glucagonlike peptide-1 7-36 amide and glucagonlike peptide-1 7-37 in healthy subjects are indistinguishable. *Diabetes 42*(5), 658–661.

Pi-Sunyer, X., Kissileff, H. R., Thornton, J., & Smith, G. P. (1982). C-terminal octapeptide of cholecystokinin decreases food intake in obese men. *Physiol Behav 29*(4), 627–630.

Plum, L., Belgardt, B. F., & Bruning, J. C. (2006). Central insulin action in energy and glucose homeostasis. *J Clin Invest 116*(7), 1761–1766.

Pocai, A., Lam, T. K., Gutierrez-Juarez, R., Obici, S., Schwartz, G. J., Bryan, J., et al. (2005). Hypothalamic K(ATP) channels control hepatic glucose production. *Nature 434*(7036), 1026–1031.

Pocai, A., Obici, S., Schwartz, G. J., & Rossetti, L. (2005). A brain-liver circuit regulates glucose homeostasis. *Cell Metab 1*(1), 53–61.

Poon, T., Nelson, P., Shen, L., Mihm, M., Taylor, K., Fineman, M., et al. (2005). Exenatide improves glycemic control and reduces body weight in subjects with type 2 diabetes: a dose-ranging study. *Diabetes Technol Ther 7*(3), 467–477.

Prodi, E., & Obici, S. (2006). Minireview: the brain as a molecular target for diabetic therapy. *Endocrinology 147*(6), 2664–2669.

Proud, C. G. (2002). Regulation of mammalian translation factors by nutrients. *Eur J Biochem 269*(22), 5338–5349.

Rahimian, R., Masih-Khan, E., Lo, M., van Breemen, C., McManus, B. M., & Dube, G. P. (2001). Hepatic over-expression of peroxisome proliferator activated receptor gamma2 in the ob/ob mouse model of non-insulin dependent diabetes mellitus. *Mol Cell Biochem 224*(1-2), 29–37.

Rapoport, S. I. (1996). In vivo labeling of brain phospholipids by long-chain fatty acids: relation to turnover and function. *Lipids*(31 Suppl), S97–S101.

Reimann, F., & Gribble, F. M. (2002). Glucose-sensing in glucagon-like peptide-1-secreting cells. *Diabetes 51*(9), 2757–2763.

Rowe, I. C., Treherne, J. M., & Ashford, M. L. (1996). Activation by intracellular ATP of a potassium channel in neurones from rat basomedial hypothalamus. *J Physiol 490*(Pt 1), 97–113.

Russek, M. (1963). Participation of hepatic glucoreceptors in the control of intake of food. *Nature 197*, 79–80.

Russek, M. (1970). Demonstration of the influence of an hepatic glucosensitive mechanism on food-intake. *Physiol Behav 5*(10), 1207–1209.

Schwartz, M. W., Peskind, E., Raskind, M., Boyko, E. J., & Porte, D., Jr. (1996). Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med 2*(5), 589–593.

Scott, V., Kimura, N., Stark, J. A., & Luckman, S. M. (2005). Intravenous peptide YY3-36 and Y2 receptor antagonism in the rat: effects on feeding behaviour. *J Neuroendocrinol 17*(7), 452–457.

Shi, H., & Bartness, T. J. (2005). White adipose tissue sensory nerve denervation mimics lipectomy-induced compensatory increases in adiposity. *Am J Physiol Regul Integr Comp Physiol 289*(2), R514–R520.

Shimazu, T. (1981). Central nervous system regulation of liver and adipose tissue metabolism. *Diabetologia*(20 Suppl), 343–356.

Smith, G. P., Jerome, C., Cushin, B. J., Eterno, R., & Simansky, K. J. (1981). Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science 213*(4511), 1036–1037.

Spanswick, D., Smith, M. A., Mirshamsi, S., Routh, V. H., & Ashford, M. L. (2000). Insulin activates ATP-sensitive K+channels in hypothalamic neurons of lean, but not obese rats. *Nat Neurosci 3*(8), 757–758.

Stanley, S., Wynne, K., & Bloom, S. (2004). Gastrointestinal satiety signals: III. Glucagon-like peptide 1, oxyntomodulin, peptide YY, and pancreatic polypeptide. *Am J Physiol Gastrointest Liver Physiol 286*(5), G693–G697.

Talsania, T., Anini, Y., Siu, S., Drucker, D. J., & Brubaker, P. L. (2005). Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. *Endocrinology 146*(9), 3748–3756.

Tanida, M., Iwashita, S., Ootsuka, Y., Terui, N., & Suzuki, M. (2000). Leptin injection into white adipose tissue elevates renal sympathetic nerve activity dose-dependently through the afferent nerves pathway in rats. *Neurosci Lett 293*(2), 107–110.

Thorens, B., & Larsen, P. J. (2004). Gut-derived signaling molecules and vagal afferents in the control of glucose and energy homeostasis. *Curr Opin Clin Nutr Metab Care 7*(4), 471–478.

Tschop, M., Smiley, D. L., & Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature 407*(6806), 908–913.

Turton, M. D., O'Shea, D., Gunn, I., Beak, S. A., Edwards, C. M., Meeran, K., et al. (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature 379*(6560), 69–72.

Uno, K., Katagiri, H., Yamada, T., Ishigaki, Y., Ogihara, T., Imai, J., et al. (2006). Neuronal pathway from the liver modulates energy expenditure and systemic insulin sensitivity. *Science 312*(5780), 1656–1659.

Verdich, C., Flint, A., Gutzwiller, J. P., Naslund, E., Beglinger, C., Hellstrom, P. M., et al. (2001). A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. *J Clin Endocrinol Metab 86*(9), 4382–4389.

Wang, R., Cruciani-Guglielmacci, C., Migrenne, S., Magnan, C., Cotero, V. E., & Routh, V. H. (2006). Effects of oleic acid on distinct populations of neurons in the hypothalamic arcuate nucleus are dependent on extracellular glucose levels. *J Neurophysiol 95*(3), 1491–1498.

Wang, R., Liu, X., Hentges, S. T., Dunn-Meynell, A. A., Levin, B. E., Wang, W., et al. (2004). The regulation of glucose-excited neurons in the hypothalamic arcuate nucleus by glucose and feeding-relevant peptides. *Diabetes 53*(8), 1959–1965.

West, D. B., Fey, D., & Woods, S. C. (1984). Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. *Am J Physiol 246* (5 Pt 2), R776–R787.

Woods, S. C. (2004). Gastrointestinal satiety signals: I. An overview of gastrointestinal signals that influence food intake. *Am J Physiol Gastrointest Liver Physiol 286*(1), G7–G13.

Woods, S. C., & Gibbs, J. (1989). The regulation of food intake by peptides. *Ann N Y Acad Sci 575*, 236–243.

Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., Murphy, K. G., et al. (2001). Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab 86*(12), 5992.

Yamada, T., & Katagiri, H. (2007). Avenues of Communication between the Brain and Tissues/Organs Involved in Energy Homeostasis. *Endocr J.*

Yamada, T., Katagiri, H., Ishigaki, Y., Ogihara, T., Imai, J., Uno, K., et al. (2006). Signals from intra-abdominal fat modulate insulin and leptin sensitivity through different mechanisms: neuronal involvement in food-intake regulation. *Cell Metab 3*(3), 223–229.

Zabolotny, J. M., Bence-Hanulec, K. K., Stricker-Krongrad, A., Haj, F., Wang, Y., Minokoshi, Y., et al. (2002). PTP1B regulates leptin signal transduction in vivo. *Dev Cell 2*(4), 489–495.

Zander, M., Madsbad, S., Madsen, J. L., & Holst, J. J. (2002). Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet 359*(9309), 824–830.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature 372*(6505), 425–432.

Zorrilla, E. P., Iwasaki, S., Moss, J. A., Chang, J., Otsuji, J., Inoue, K., et al. (2006). Vaccination against weight gain. *Proc Natl Acad Sci U S A 103*(35), 13226–13231.

Glycoconj J
DOI 10.1007/s10719-007-9102-8

Design and synthesis of versatile ganglioside probes for carbohydrate microarrays

Akihiro Imamura · Takeru Yoshikawa · Tatsuya Komori · Masatoshi Ando · Hiromune Ando · Masahiro Wakao · Yasuo Suda · Hideharu Ishida · Makoto Kiso

Received: 2 November 2007 / Revised: 25 December 2007 / Accepted: 26 December 2007

Abstract A series of ganglioside GM1-, GM2-, and GM3-type probes, in which the ceramide portion is replaced with a glucose residue, were systematically synthesized based on a convergent synthetic method.

Keywords Chemical synthesis · Gangliosides · Glycosylation · Carbohydrate probe

Introduction

Gangliosides, anionic glycosphingolipids with various sugar chains containing one or more residues of sialic acid, exist universally on cell surface. They participate in vital processes, such as immune or nervous systems, as molecules responsible for cell–cell and cell–ligand interactions [1, 2]. In particular, a series of gangliosides, such as GM1, GM2 and GM3, are important as regulatory factors for the differentiation of the central nervous system and serve as cell-attachment receptors for some viruses, bacteria and bacterial toxins [3, 4]. Moreover, many profound relationships between those gangliosides and a number of cancers and diseases have been demonstrated [5, 6]. However, the biological functions of gangliosides are not fully understood, due to their structural complexities and the low affinities of interaction with ligands, despite numerous studies conducted to date. To solve these issues, a considerable number of efforts have gone into the development of analytical techniques for sensitive detection of carbohydrate–ligand interactions. Consequently, many carbohydrate microarray technologies have been developed to facilitate glycomics research [7]. Coincidentally, many carbohydrate probes that incorporate specific functional groups such as azide [8], thiol [9] and maleimide [10] have been chemically synthesized for the fabrication of microarrays. Recently, oligosaccharide-immobilized chips (named Sugar_Chips), which provide real-time and high-throughput analysis of oligosaccharide–protein interaction without any labeling of the targeted protein, have been developed [11], in which chemically synthesized oligosaccharides having D-glucose, which provides a reactive aldehyde functionality, at the reducing end were used. The D-glucose residue also serves as a spacer between a targeted sugar chain and a scaffold for immobilization, because of its appropriate hydrophilicity and flexibility. Furthermore, it has been demonstrated that a reducing sugar directly participates in the noncovalent link to a scaffold [12, 13]. Accordingly, as exemplified in Fig. 1, the chemically synthesized oligo-

A. Imamura (✉) · T. Yoshikawa · T. Komori · M. Ando · H. Ishida · M. Kiso (✉)
Department of Applied Bioorganic Chemistry,
Faculty of Applied Biological Sciences, Gifu University,
1-1 Yanagido, Gifu-shi, Gifu 501-1193, Japan
e-mail: gif012@gifu-u.ac.jp
e-mail: kiso@gifu-u.ac.jp

H. Ando
Division of Instrumental Analysis, Life Science Research Center,
Gifu University, 1-1 Yanagido, Gifu-shi,
Gifu 501-1193, Japan

M. Wakao · Y. Suda
Department of Nanostructure and Advanced Materials,
Graduate School of Science and Engineering and Venture
Business Laboratory, Kagoshima University,
Kohrimoto, Kagoshima 890-0065, Japan

M. Kiso
Institute for Integrated Cell-Material Sciences (iCeMS),
Kyoto University,
Kyoto, Japan

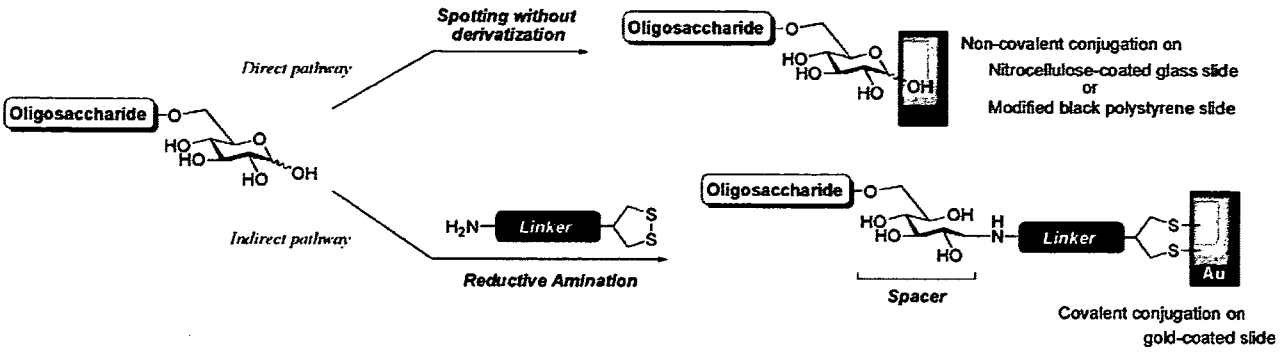


Fig. 1 Two examples for carbohydrate microarray fabrication

saccharide probes are expected to be immobilized by the direct and indirect attachment to scaffolds. We report here the facile synthesis of glucose-ended probes of ganglioside GM1, GM2, and GM3 for carbohydrate microarrays (Fig. 2).

Results and discussion

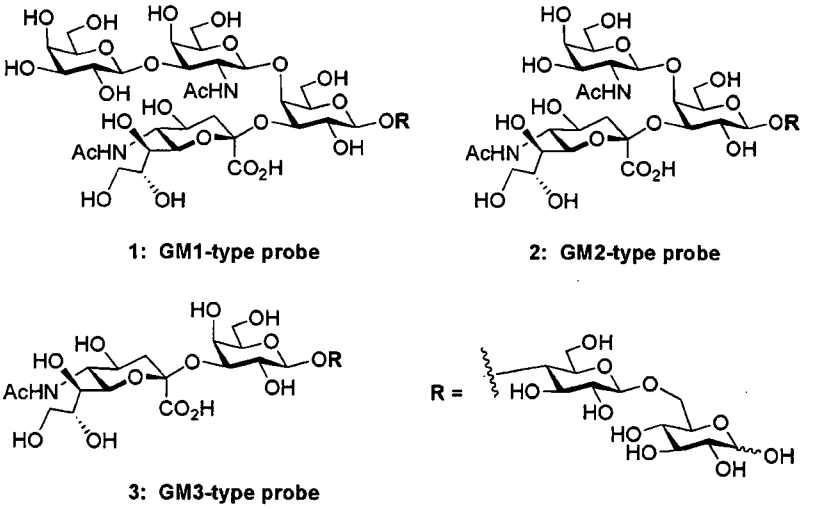
Taking a look at target molecules, we have hypothetically disconnected them into two parts: common sequence SAα(2→3)Galβ(1→4)Glcβ(1→6)Glc, and the other sugar parts. The common sequence was further disconnected at Galβ(1→4)Glc linkage, providing SAα(2→3)Gal and gentiobiose segments, based on the recently reported efficient syntheses of GM2 analogs [14]. Considering the difficulty to fashion a branch out from galactose residue, the incorporation of GalN parts into Gal residue was planned to be conducted earlier than that of gentiobiose as depicted in Fig. 3.

According to our previous report [14], 2,6-*O*-dibenzylated galactoside was efficiently sialylated at C-3 position with *N*-Troc-protected sialyl donor [15, 16], producing a key sialyl galactoside **4**, which can be obtained in a crystalline form after rough chromatographic purification of the reaction mixture (Fig. 4).

The disaccharide **4** was coupled with Galβ(1→3)GalN **6** [17] or GalN donor **5** in the presence of NIS and TfOH [18] to afford the GM2-core trisaccharide **7** in 97% yield and the GM1-core tetrasaccharide **8** in 89% yield, respectively, as depicted in Table 1.

A series of ganglioside-core frames **4**, **7**, and **8** were converted into the corresponding glycosyl donors **13**, **14**, and **15**, respectively. The selective removal of the Troc group of **4** by the action of zinc–copper couple [19, 20] in acetic acid/1,2-dichloroethane at 40°C proceeded smoothly to give a free amino derivative, which, on successive treatment with acetic anhydride in pyridine afforded the corresponding *N*-acetyl derivative **9**. The use of 1,2-dichloroethane (DCE) was critical for an efficient reduction of Troc group; otherwise the reaction was sluggish. Initially, we were afraid that DCE as solvent itself consumes zinc–copper couple as reductant. Though it is not clear whether DCE is advantageous for electron transfer from zinc–copper couple, we were intriguingly able to observe smooth proceeding of the reaction in a single liquid

Fig. 2 Structure of synthetic ganglioside probes



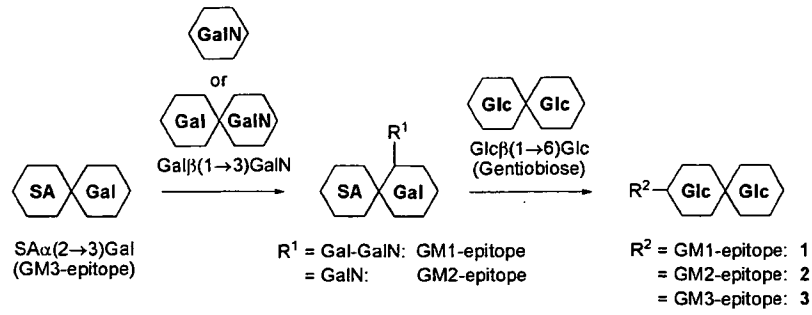


Fig. 3 Systematic reaction scheme for preparation of the reductive glucose-functionalized ganglioside probes

phase within a short time. The cleavage of benzyl groups was executed by hydrogenolysis and the following benzoylation of the resulting hydroxyl groups gave **11**. Libration of the anomeric hydroxyl group of **11** was achieved by treatment with ceric ammonium nitrate (CAN) in acetonitrile–toluene–water (6:5:3) [21]. The obtained hemiacetal was then converted into the β-trichloroacetimidate **13**, which was ready for the final glycosylation with the gentiobiose acceptor **21** as mentioned hereinafter. Interestingly, the use of less than a stoichiometric amount of DBU resulted in the predominant formation of the β-imidate derivative. The conversion of **7** and **8** into the corresponding donor **14** and **15** were also achieved by similar procedure, respectively. (Scheme 1)

Scheme 2 shows the preparation of the gentiobiose acceptor **21** as the common synthetic block, which was expected to have an enhanced reactivity at C-4 hydroxyl due to the effect of electron-donating benzyl groups. Coupling of the known glucose donor **16** [22] and acceptor **17** [23] was conducted in the presence of NIS and TfOH in CH_2Cl_2 at 0°C to give the disaccharide **18** in 90% yield. The β-configuration of the newly formed intersaccharide linkage between **16** and **17** is apparent from the relatively large coupling constant (8.2 Hz) between H-1′ and H-2′ in ^{1}H NMR spectra. Removal of the benzoyl groups under conventional conditions and benzylation of the hydroxyl groups gave **20** with a yield of 88% in two steps. Finally, reductive opening of the benzylidene group was achieved by a treatment with triethylsilane and $BF_3{\cdot}OEt_2$ in CH_2Cl_2 [24] to afford **21** with a yield of 85%.

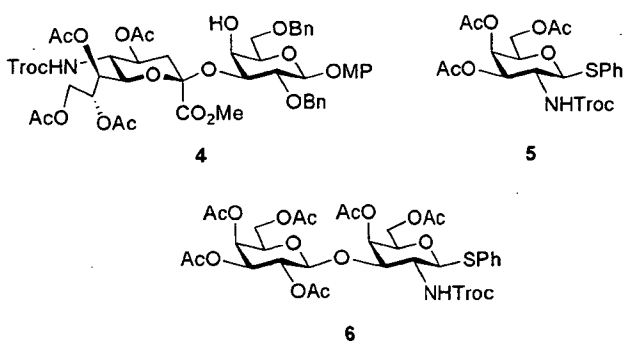


Fig. 4 Structure of glycosyl acceptor (**4**) and donors (**5**, **6**)

Scheme 3 incorporates final glycosylations of **21** with a series of ganglioside-core donors, **13**, **14**, and **15** in the presence of TMSOTf in CH_2Cl_2 at 0°C. The β-imidate **13** was coupled with the gentiobiose acceptor **21** by treatment with TMSOTf at 0°C to afford the desired β-glycoside **22** in an excellent yield. The α-imidate **14** and **15** were subjected to the glycosidation with **21** under essentially the same conditions for **13** to give **23** and **24** in good yields, respectively. Finally, global deprotection of the above-mentioned glycans was conducted. After de-acylation under Zemplén conditions and subsequent saponification of the fully protected oligosaccharides, **24**, **23**, and **22**, hydrogenolysis for each resultant compound was performed in the presence of $Pd(OH)_2/C$ under H_2 atmosphere to afford the target carbohydrate probes **1**, **2** and **3** in good to excellent yields, respectively.

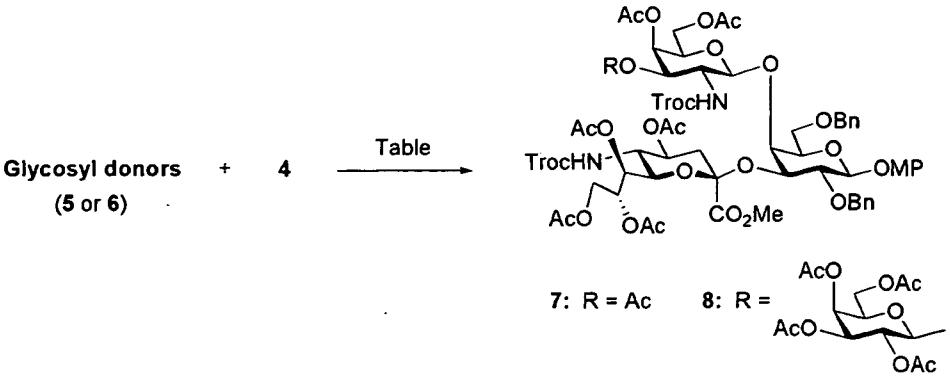
In conclusion, we have succeeded in the synthesis of ganglioside GM1-, GM2-, and GM3-type probes for carbohydrate microarray analyses. It was found that the convergent synthetic strategy between the defined ganglioside-core frame and the reducing end glucose can be used for the synthesis of complex ganglioside probes. In addition, synthesized ganglioside probes are currently used as one of the oligosaccharide probes on immobilized-chips by Suda's group. We are currently underway to expand the existing pool of functional carbohydrate probes containing more complex gangliosides.

Experimental

General procedures

All reactions were carried out under a positive pressure of argon, unless otherwise noted. All chemicals were purchased from commercial suppliers and used without further purification, unless otherwise noted. Molecular sieves were purchased from Wako Chemicals Inc. and dried at 300°C for 2 h in muffle furnace prior to use. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Inova 400/500 spectrometer and a JEOL ECA 500/600 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Data are presented as

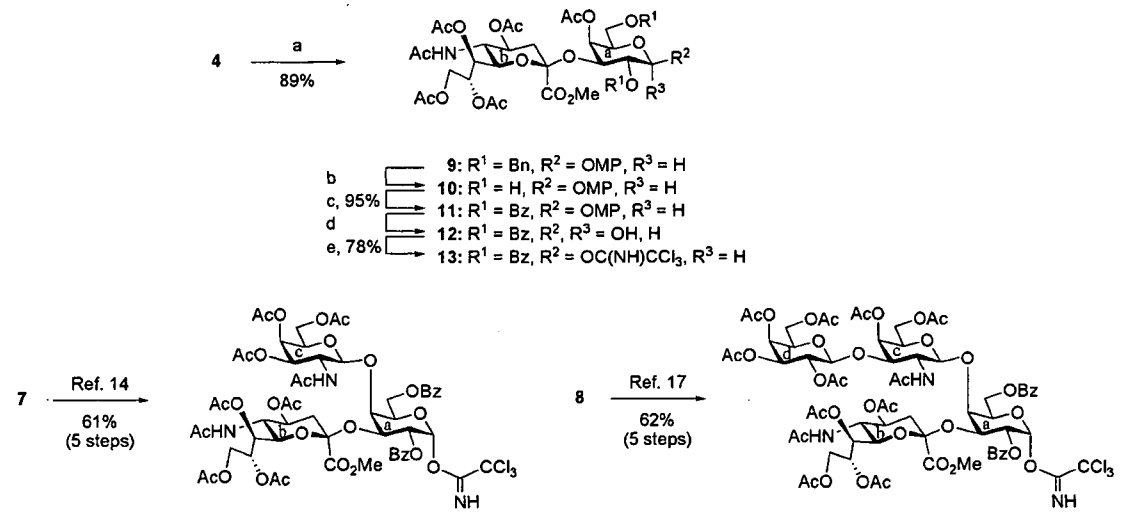
Table 1 Glycosylation of **4** with glycosyl donors **5** and **6**



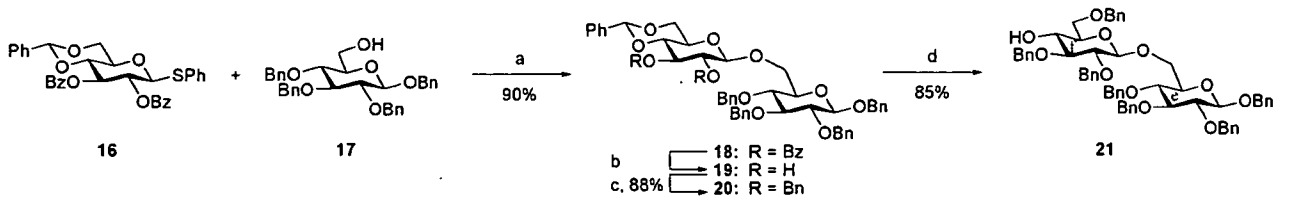
Entry	Donor	Condition	Temp.[°C]	Product	% Yield
1	**5**	NIS TfOH MS4Å CH_2Cl_2	0	**7**	97
2	**6**		-40	**8**	89

follows: Chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, dd=double of doublet, m=multiplet and/or multiple resonances), integration, coupling constant in Hertz (Hz). MALDI-TOF MS spectra were recorded in the positive ion mode on a Bruker Autoflex with the use of α-cyano-4-hydroxy-cinnamic acid (CHCA) as a matrix. Optical rotations were measured with a 'Horiba SEPA-300' polarimeter. Column chromatography was performed on silica gel (Fuji Silysia Co., 80 and 300 mesh). Reactions were monitored by TLC on silica gel $60F_{254}$ (Merck, glass plate) and the compounds were detected by examination under UV light (2,536 Å) and visualized by dipping the plates in a 10% sulfuric acid–ethanol solution or 20% phosphomolybdic acid–ethanol solution followed by heating. Organic solutions were concentrated by rotary evaporation below 45°C under reduced pressure. Solvent systems in chromatography were specified in *v/v*.

4-Methoxyphenyl {methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)}-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (**9**) To a solution of compound **4** (500 mg, 465 μmol) in 1,2-dichloroethane (6.1 ml) were added acetic acid (18.3 ml) and zinc–copper couple (2.50 g). The mixture was stirred for 1.5 h at 40°C, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH=15:1). The



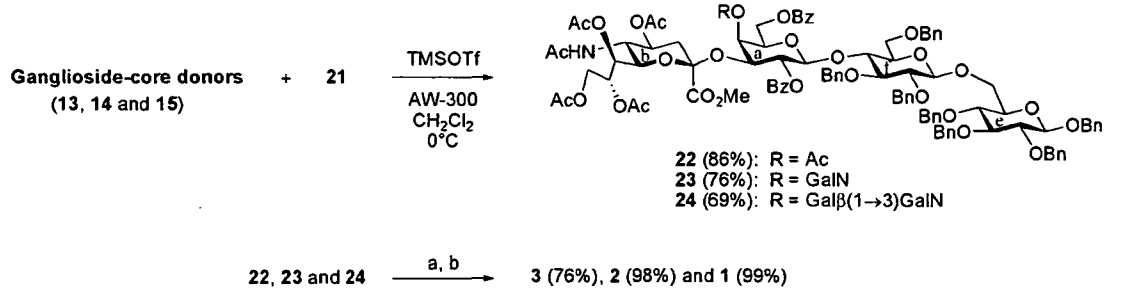
Scheme 1 Conversion of ganglioside-core frames to the corresponding glycosyl donors. Reagents and conditions: *a* Zn-Cu, AcOH, 1,2-DCE, 40°C then Ac_2O, Py; *b* $Pd(OH)_2/C$, H_2, EtOH; *c* Bz_2O, Py; *d* CAN, CH_3CN-PhMe-H_2O (6/5/3); *e* CCl_3CN, DBU, CH_2Cl_2, 0°C



Scheme 2 Preparation of the gentiobiosyl acceptor **21**. Reagents and conditions: *a* NIS, TfOH, MS4Å, CH_2Cl_2, 0°C; *b* NaOMe, MeOH-THF (2/1); *c* BnBr, NaH, DMF; *d* TESH, $BF_3{\cdot}OEt_2$, CH_2Cl_2

reaction mixture was filtered through Celite. The combined filtrate and washings was extracted with $CHCl_3$, and the organic layer was washed with H_2O, sat. Na_2CO_3, and brine, dried over Na_2SO_4 and concentrated. To a solution of the residue in pyridine (5.0 ml) was added acetic anhydride (2.5 ml). The mixture was stirred for 13 h at ambient temperature, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH=15:1). The reaction mixture was coevaporated with toluene and extracted with $CHCl_3$. The organic layer was washed with 2 M HCl, H_2O, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography on silica gel (EtOAc/hexane=3:1) to give **9** (406 mg, 89%).; $[\alpha]_D$=−15.4° (*c* 0.9, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.45–6.77 (m, 14 H, 2 Ph and 1 MP), 5.53 (m, 1 H, H-8b), 5.33 (dd, 1 H, H-7b), 5.24 (d, 1 H, $J_{5,NH}$=8.9 Hz, NH), 5.07 (m, 2 H, H-1a, 4a), 4.96–4.88 (m, 3 H, H-4b, 2 CH*H*Ph), 4.63 (dd, 1 H, H-3a), 4.53 (d, 1 H, CH*H*Ph), 4.46 (d, 1 H, CH*H*Ph) 4.36 (dd, 1 H, H-9'b), 4.13 (q, 1 H, $J_{5,NH}$= 8.9 Hz, H-5b), 3.96–3.94 (m, 2 H, H-6'a, 9b), 3.85 (s, 3 H, OMe), 3.76–3.73 (m, 5 H, H-2a, 6b, OMe), 3.56–3.52 (m, 2 H, H-5a, 6a), 2.63 (dd, 1 H, H-3b$_{eq}$), 2.12–1.83 (m, 19 H,6 Ac, H-3b$_{ax}$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.9, 170.6, 170.3, 170.2, 170.0, 168.1, 155.1, 151.7, 139.4, 138.0, 128.3, 128.1, 127.7, 127.6, 127.1, 118.2, 114.4, 102.4, 97.1, 78.1, 74.8, 73.5, 73.1, 72.3, 72.2, 69.5, 68.9, 68.7, 68.6, 67.2, 62.2, 55.6, 53.1, 49.2, 37.6, 23.2, 21.3, 20.8, 20.8, 20.5; MALDI MS: *m/z*: calcd for $C_{49}H_{59}O_{20}NNa$: 1,004.35; found: 1,004.35 [*M* + Na] $^+$.

4-Methoxyphenyl {methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)}-4-O-acetyl-2,6-di-O-benzoyl-β-D-galactopyranoside (**11**) To a solution of compound **9** (385 mg, 392 μmol) in EtOH (30 ml) was added palladium hydroxide [$Pd(OH)_2$] (20 wt% Pd on carbon; 400 mg). The mixture was vigorously stirred for 4 h at ambient temperature under hydrogen atmosphere, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH=15:1). The reaction mixture was filtered through Celite. The combined filtrate and washings was concentrated. To a solution of the residue in pyridine (5.0 ml) was added benzoic anhydride (354 mg, 1.57 mmol). The mixture was stirred for 16 h at ambient temperature, as the proceeding of the reaction was monitored by TLC ($CHCl_3$/MeOH=15:1). The reaction mixture was coevaporated with toluene and extracted with $CHCl_3$. The organic layer was washed with 2 M HCl, H_2O, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography on silica gel (EtOAc/hexane=3:1) to give **11** (380 mg, 95%); $[\alpha]_D$=+ 27.9° (*c* 4.2, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.17–6.67 (m, 14 H, 2 Ph and 1 MP), 5.59 (m, 1 H, H-8b), 5.55 (t, 1 H, $J_{1,2}$=8.3 Hz, $J_{2,3}$=10.3 Hz, H-2a), 5.26 (d, 1 H, $J_{1,2}$=8.3 Hz, H-1a), 5.20 (dd, 1 H, $J_{6,7}$=2.8 Hz, H-7b), 5.16 (d, 1 H, $J_{3,4}$=3.4 Hz, H-4a), 5.14 (d, 1 H, NH), 4.87 (dd, 1 H, $J_{2,3}$=10.3 Hz, $J_{3,4}$=3.4 Hz, H-3a), 4.85 (m, 1 H, H-4b), 4.46 (t, 1 H, H-6'a), 4.35 (dd, 1 H, H-6a), 4.27 (dd, 1 H, H-9'b), 4.19 (t, 1 H, H-5b), 3.91 (dd, 1 H, H-9b), 3.86–3.79 (m, 4 H, H-5b, OMe) 3.71 (s, 3 H, OMe), 3.61 (dd, 1 H, $J_{6,7}$=2.8 Hz,



Scheme 3 Coupling of the ganglioside-core donors (**13**, **14** and **15**) and the gentiobioside acceptor (**21**), and subsequent global deprotections. Reagents and conditions: *a* NaOMe, MeOH, 45°C or reflux, then H_2O; (b) $Pd(OH)_2$/C, H_2, H_2O or MeOH-H_2O (5/2), RT or 40°C