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Histamine Regulation in Glucose and Lipid Metabolism via Histamine Receptors

Model for Nonalcoholic Steatohepatitis in Mice

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Histamine has been proposed to be an important regulator of energy intake and expenditure. The aim of this study was to evaluate histamine regulation of glucose and lipid metabolism and development of nonalcoholic steatohepatitis (NASH) with a hyperlipidemic diet. Histamine regulation of glucose and lipid metabolism, adipocytokine production, and development of hyperlipidemia-induced hepatic injury were studied in histamine H1 ($H1R^{-/-}$) and H2 ($H2R^{-/-}$) receptor knockout and wild-type mice. $H1R^{-/-}$ mice showed mildly increased insulin resistance. In contrast, $H2R^{-/-}$ mice manifested profound insulin resistance and glucose intolerance. High-fat/high-cholesterol feeding enhanced insulin resistance and glucose intolerance. Studies with two-deoxy-2-[^{18}F]-fluoro-D-glucose and positron emission tomography showed a brain glucose allocation in $H1R^{-/-}$ mice. In addition, severe NASH with hypoadiponectinemia as well as hepatic triglyceride and free cholesterol accumulation and increased blood hepatic enzymes were observed in $H2R^{-/-}$ mice. $H1R^{-/-}$ mice showed an obese phenotype with visceral adiposity, hyperleptinemia, and less severe hepatic steatosis and inflammation with increased hepatic triglyceride. These data suggest that H1R and H2R signaling may regulate glucose and lipid metabolism and development of hyperlipidemia-induced NASH. ***(Am J Pathol 2010, 177:713–723; DOI: 10.2353/ajpath.2010.091198)***

Histamine, one of the mediators of inflammation and immunity, is produced from L-histidine by the rate-limiting enzyme histidine decarboxylase (HDC). HDC is expressed in various types of cells, including mast cells, monocytes/macrophages, T lymphocytes, enterochromaffin-like cells, and neuronal cells.[1–3] The effects of histamine are mediated through specific histamine receptors, which have been classified into the H1, H2, H3, and H4 subtypes.[4] The recent development of gene-modified mice lacking HDC or histamine receptors has provided valuable tools to analyze the functions of histamine.[5] For example, HDC knockout (KO) mice were reported to show clinical features of visceral adiposity, hyperleptinemia, and decreased glucose tolerance.[6] In addition, it was reported that $H1R^{-/-}$ mice fed a high-fat diet showed increased fat deposition and leptin resistance and that disruption of the H3R gene in mice resulted in an obese phenotype and glucose intolerance, with elevated blood insulin and leptin levels.[7,8] Taken

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together, these studies indicate that histamine plays important roles in energy regulation and metabolism.

In contrast, insulin resistance/metabolic syndrome, which increases the risk for atherosclerosis and cardiovascular events, is an aggregate of disorders related to obesity or visceral adiposity, insulin resistance, hyperlipidemia, and hypertension.[9,10] Recently, the liver has also been recognized as one of the pathological targets of metabolic syndrome.[11,12] Insulin resistance is associated with increased adiposity and nonalcoholic fatty liver disease, which can lead to the advanced condition known as nonalcoholic steatohepatitis (NASH). Typical morphological features of NASH in humans include steatosis, inflammation and pericellular fibrosis.[13] Obese and diabetic *ob/ob* mice develop steatohepatitis, but not fibrosis, in their liver.[14] Other obese and diabetic db/db mice fed a diet lacking methionine and choline exhibited liver fibrosis as a model of NASH.[15] Adiponectin, an adipocytokine secreted from adipocytes,[16,17] was recently reported to be centrally involved in the pathogenesis of metabolic syndrome and nonalcoholic fatty liver disease.[18,19]

Because the loss of histamine functions has been suggested to result in disturbed energy regulation and metabolism,[6–8] we speculated that the functions of histamine might be closely related to the pathogenesis of insulin resistance syndrome, metabolic disturbances, and NASH. In this study, we evaluated the phenotypic differences of wild-type, $H1R^{-/-}$, and $H2R^{-/-}$ mice in terms of glucose metabolism, lipid metabolism, and the expression of adipocytokines, including adiponectin and leptin after high-fat/high-cholesterol diet (HcD). In addition, we examined *in vivo* glucose uptake using 2-deoxy-2-[^{18}F]-fluoro-D-glucose ([^{18}F]FDG) and positron imaging of whole mice bodies.

Materials and Methods

Animals and Diets

Targeting vectors were constructed in pMC1 ($H1R^{-/-}$) and pPNT ($H2R^{-/-}$) vectors and transfected into E14 ES cells. After the *neo*^{r} colonies were selected and verified by PCR and Southern blotting, the targeted ES cells were injected into blastocysts from C57BL/6 mice. The resulting male chimeras were mated with C57BL/6 mice to generate heterozygous mice.[20,21] Experiments were performed on 8-week-old male wild-type, $H1R^{-/-}$, and $H2R^{-/-}$ mice (backcrossed for nine generations) weighing 20–25 g (7–30 mice per experiment). Mice were fed a normal chow diet (NcD) or a HcD (1.25% cholesterol, 0.5% cholic acid, and 15% lard) for 14 weeks. On a caloric basis, HcD consisted of 41.5% fat from lard, 40.8% carbohydrate, and 17.7% protein (total, 4.44 kcal/g). Mice were euthanized by an overdose of pentobarbital (i.p.) at the indicated periods of time. All protocols were approved by the Ethics Committee of Animal Care and Experimentation, University of Occupational and Environmental Health, and were performed according to the Institutional Guidelines for Animal Experiments and to Law (number 105) and Notification (number 6) of the Japanese government.

Analysis of Glucose Metabolism

To examine the presence of glucose intolerance, mice were fasted for 16 hours and then injected (i.p.) with glucose at a dose of 1 mg/g body weight. Blood samples were collected from the tail vein at 0, 30, 60, and 120 minutes after the glucose injection. To determine insulin tolerance, mice were injected (i.p.) with human insulin (1 U/kg body weight), and blood samples were collected at 0, 30, 60, and 120 minutes after the insulin injection. Blood glucose levels were measured using a One-Touch glucose monitoring system (Bayer Medical, Tokyo, Japan). Blood insulin levels were also measured by an enzyme-linked immunosorbent assay (Morinaga, Tokyo, Japan) from the same samples obtained after the glucose challenge test according with the manufacturer's instructions.

Blood Levels of Adiponectin and Leptin

Blood levels of adiponectin and leptin were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions.

Real-Time PCR

After extracting total RNA from the liver, skeletal muscle and visceral adipose tissue using TRIzol reagent (Life Technologies, Grand Island, NY), the mRNA expression of glucose transporters (Glut), adiponectin receptor (AdipoR), sterol-responsive element-binding protein-1b/c and 2 (SREBP-1 and -2), hydroxymethylglutaryl-CoA reductase (HMGR) and fatty acid synthase (FASn) was analyzed by real-time PCR (TaqMan probe) using the primer pairs summarized in Table 1. The levels of mRNA expression were normalized by those of the expression of 18s ribosomal RNA in the same samples.

Western Blotting of FASn

Fresh frozen liver tissues were lysed with a lysis buffer (10 mmol/L Tris-HCl [pH 7.5], 0.4 M NaCl, 1% Triton X-100, 0.1% Nonidet P-40, and 1 mmol/L phenylmethylsulfonyl fluoride) and applied to SDS-PAGE and electrotransfer onto polyvinylidene difluoride membrane. The expression of hepatic FASn was detected using anti-FASn antibody (American Research Products, Belmont, MA).

Dissection Analysis of [^{18}F]FDG in Mice

The production of [^{18}F]FDG was performed according to a method described elsewhere.[22] [^{18}F]FDG (2.5 MBq) was injected into mice through the tail veins. The mice were then sacrificed by decapitation under chloral hydrate anesthesia 45 minutes after the injection. Blood