SHORT COMMUNICATION

Autocrine Effects of Visfatin on Hepatocyte Sensitivity to Insulin Action

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Summary
Visfatin was originally described as an adipokine with insulin mimetic effects. Recently, it was found that visfatin is identical with the Nampt (nicotinamide phosphoribosyltransferase) gene that codes for an intra- and extracellular NAD biosynthetic enzyme and is predominantly expressed outside the adipose tissue. In the current study, we found strong protein and mRNA expression of visfatin in rat heart, liver, kidney, and muscle, while the expression of visfatin in visceral fat was significantly lower and undetectable in subcutaneous fat. The insulin-mimetic effects of visfatin (extracellular form of Nampt or eNampt) are controversial and even less is known about autocrine effects of visfatin (intracellular form of Nampt or iNampt). Since liver plays a major role in glucose metabolism, we studied visfatin effects on insulin-stimulated cellular glucose uptake in Fao rat hepatocytes using RNA interference (RNAi). RNAi-mediated downregulation of visfatin expression in Fao cells was associated with significantly reduced NAD biosynthesis (0.3±0.01 vs. 0.5±0.01 mmol/h/g, P<0.05) and with significantly decreased incremental glucose uptake after stimulation with insulin when compared to controls with normal expression of visfatin (0.6±0.2 vs. 2.2±0.5 nmol/g/2 h, P=0.02). These results provide evidence that visfatin exhibits important autocrine effects on sensitivity of liver cells to insulin action possibly through its effects on NAD biosynthesis.

Key words
Visfatin • Fao hepatocytes • Insulin resistance • RNA interference

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Adipokines are bioactive mediators released from adipose tissue. The effects of adipokines on vascular function, immune system regulation and adipocyte metabolism make them key players in the pathogenesis of metabolic syndrome (Rajala et al. 2003). Recently, Fukuhara et al. (2005) reported identification of a new adipokine named visfatin (visceral fat adipokine). Several subsequent studies have focused on clarifying the expression, secretion and function of visfatin in glucose and lipid metabolism, but with conflicting results (Sethi et al. 2005, Arner 2006, Brendt et al. 2006, Chen et al. 2006, Stephens et al. 2006). Later on, visfatin was found to be identical with the presumptive cytokine PBEF (pre-B cell colony-enhancing factor) (Samal et al. 1994) and finally with the Nampt (nicotinamide phospho-ribosyltransferase) gene that is expressed predominantly outside the adipose tissue and that codes for an enzyme involved in nicotinamide adenine dinucleotide (NAD) biosynthesis.
(Rongvaux et al. 2002). Recently, Revollo et al. (2007) demonstrated that extracellular visfatin (eNampt) does not exert insulin-mimetic effects in vitro when exogenously administered visfatin showed no effects on differentiation of the human SGBS preadipocyte cell line or on glucose uptake in 3T3-L1 adipocytes. Fukuhara et al. (2005) reported insulin mimetic effects of visfatin also in liver cells when exogenous visfatin suppressed glucose release in H4IIEC3 hepatocytes. In the current study, we analyzed the expression of visfatin in rat tissues and studied visfatin effects on cellular glucose uptake and insulin sensitivity in Fao rat hepatocytes using RNA interference (RNAi)-mediated down regulation of visfatin expression.

Figures 1A and 1B show the expression of visfatin mRNA and protein in tissues isolated from Wistar-Kyoto (WKY) rats detected by agarose gel electrophoresis of the PCR products through reverse transcription of the total RNA and by Western blot analysis. As can be seen, no visfatin-specific PCR product was detected in the subcutaneous fat tissue. The absence of visfatin protein in subcutaneous fat tissue was also confirmed by Western blot analysis (Fig. 1B). Visfatin expression was significantly increased in heart, liver and kidney compared to muscle, brain, and visceral fat. These expression results in WKY rats are in agreement with those reported by Revollo et al. (2007) in the mouse.
To test possible autocrine effects of visfatin (iNampt) on glucose uptake and insulin sensitivity in liver cells, we analyzed whether downregulation of visfatin expression by RNAi could affect glucose incorporation into Fao hepatocytes and how this will be modified in the presence of insulin. The efficiency of the silencing of the visfatin gene expression by RNAi was evaluated at the protein level by Western blot analysis and at the mRNA level by qRT-PCR. Experiments included untreated control cells, and as a negative control, cells transfected with a nonsense sequence. Figures 1C and 1D show significantly decreased levels of both RNA and protein in transfected Fao hepatocytes confirming significant downregulation of visfatin. The role of visfatin in cellular glucose uptake was analyzed by measuring incorporation of radioactively labeled 14C-glucose into Fao hepatocytes in the presence or absence of insulin in the incubation media. Figure 2A shows that Fao hepatocytes with downregulated visfatin expression exhibited significantly reduced levels of cellular glucose uptake and were insulin resistant because the incremental increase in glucose incorporation after insulin stimulation was significantly lower compared to control hepatocytes (0.6±0.2 vs. 2.2±0.5 mmol/g/2 h, P=0.02). These results indicate that autocrine effects of visfatin may play an important role in regulating insulin sensitivity in the liver. It can be hypothesized that insulin-sensitizing effects of visfatin are related to its essential role in the NAD biosynthetic pathway. Accordingly, we also tested whether RNAi induced downregulation of visfatin affects NAD biosynthesis. As can be seen in Figure 2B, NAD biosynthesis rate was significantly reduced in the Fao hepatocytes with downregulated levels of visfatin. These results provide evidence that visfatin exhibits important autocrine effects on sensitivity of liver cells to insulin action possibly through its effects on NAD biosynthesis.

Conflict of Interest
There is no conflict of interest.

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