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Research report

Evidence for the involvement of neuropeptide Y in the antidepressant effect of imipramine in type 2 diabetes

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ABSTRACT

Depression is a major comorbidity factor of diabetes and the outcome of one disorder influences the other. Our aim is to scrutinize the link between the two, if any. Since neuropeptide Y (NPY) system plays an important role in regulating central glucose sensing mechanisms, and also depression-related behavior, we test the involvement of NPY in the modulation of depression in type 2 diabetic mice. The mice were fed on high-fat diet and administered with low dose of streptozotocin to induce type 2 diabetes. These animals showed augmented plasma glucose and increased immobility time in tail suspension test (TST) suggesting induction of diabetes and depression. Intracerebroventricular (icv) treatment with NPY or NPY Y1 receptor agonist [Leu^{31}, Pro^{34}]-NPY and intraperitoneal treatment with imipramine decreased immobility time. However, opposite effect was produced by NPY Y1 receptor antagonist BIBP3226 (icv). Moreover, reduced immobility time by imipramine was potentiated by NPY and [Leu^{31}, Pro^{34}]-NPY, but attenuated by BIBP3226. Immunohistochemical analysis of the different nuclei of the extended amygdala, the region primarily involved in affective disorders, was undertaken. A significant reduction in NPY immunoreactivity in the central nucleus of amygdala, nucleus accumbens shell and lateral division of bed nucleus of stria terminalis of the diabetic mice was noticed; the response was ameliorated in imipramine treated animals. The results suggest that decreased NPY expression in the extended amygdala might be causally linked with the depression induced following type 2 diabetes and that the antidepressant action of imipramine in diabetic mice might be mediated by NPY-NPY Y1 receptor system.

1. Introduction

According to the report of World Health Organization (November 2014), 347 million people worldwide suffer from diabetes and it will be the seventh leading cause of death in 2030. Diabetes is also causal to a range of secondary manifestations like cardiovascular diseases, blindness, kidney failure, impotence and lower limb amputation. In addition, severe negative consequences like depression are commonly encountered in the patients suffering from type 2 diabetes (T2D) (Lustman et al., 2000; de Groot et al., 2001). Indeed, about 70% of T2D patients showed prevalence of depression (Shah et al., 2008; Iype et al., 2009). Patients with diabetes and depression usually show poor self-management / medication adherence resulting in higher health care costs, disability and mortality rates, and 10-fold increase in risk of suicide or suicidal ideation (Joseph et al., 2013). Interestingly, the opposite is also true, depressed patients are more susceptible to T2D (Krishnan and Nestler, 2008). Several studies underscore a close co-

Abbreviations: 3V, third ventricle; ac, anterior commissure; aca, anterior part anterior commissure; AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; acp, posterior part anterior commissure; ANOVA, analysis of variance; ARC, hypothalamic arcuate nucleus; AUC, area under the curve; BIBP3226, N2-diphenylacetyl-N- [(4-hydroxy-phenyl)-methyl]-D-arginine amide; aCSF, artificial cerebrospinal fluid; BLA, anterior part of basolateral amygdaloid nucleus; BNSTl, lateral division of the bed nucleus of stria terminalis; BSTMV, ventral part of medial division of the bed nucleus of stria terminalis; CeA, central nucleus of amygdala; HFD, high-fat diet; Icv, intracerebroventricular; IMPR, imipramine; Ip, intraperitoneal; LP-NPY, [Leu^{31}, Pro^{34}]-NPY; LSV, ventral part of lateral septal nucleus; LV, lateral ventricle; MePD, posterodorsal part of medial amygdaloid nucleus; NPY, neuropeptide Y; opt, optic tract; PBS, phosphate-buffered saline; PS, parastrial nucleus; S.E.M., standard error of mean; StA, strial part preoptic area; STZ, streptozotocin; T2D, type 2 diabetes; TST, tail suspension test; VP, ventral pallidum

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occurrence of the two conditions. Recently, Gupta et al. (2014) reported depression-like behavior in diabetic rats, which was associated with decreased serotonin levels in the brain, a hallmark of depression. Serotonin also mediates the antidepressant effect of neuropeptide Y (NPY) (Redrobe et al., 2005), while reduced levels of serotonin and NPY were associated with depression (Luo et al., 2008). These data raise a possibility that NPY may play an important role in the neurobiology of diabetes-induced depression.

NPY in the hypothalamus has emerged as a potent orexigenic agent (Nakhate et al., 2009; Taksande et al., 2011; Sohn, 2015). However, it is also known to exert antidepressant and anxiolytic-like effects in the framework of extended amygdala (Deo et al., 2010; Desai et al., 2014; Antunes et al., 2015; Ozsoy et al., 2016). Patients of major depression and those with a suicidal tendency show decreased level of NPY in the brain (Widerlov et al., 1986; Widdowson et al., 1992). Similarly, cerebrospinal fluid concentrations of NPY were decreased in depressed patients (Gjerris et al., 1992). NPY immunoreactivity and NPY Y1 receptors populations were reduced in the limbic regions of Flinders Sensitive Line rats; a genetic animal model of depression (Overstreet, 1993; Overstreet et al., 1995; Caberlotto et al., 1999). Depression induced by cholecystokinin-4 was attributed to the decreased NPY levels in the extended amygdala (Desai et al., 2014). In mice, central administration of NPY and [Leu^{31}, Pro^{34}]-NPY (NPY Y1 receptor agonist) produced antidepressant-like effect (Goyal et al., 2006, Desai et al., 2014), which was attenuated by prior treatment with BIBP3226 (NPY Y1 receptor antagonist) (Rudolf et al., 1994; Doods et al., 1996). Available data also suggest a role for NPY in the regulation of glucose sensing. Glucose-sensitive NPY neurons in the arcuate nucleus of hypothalamus (ARC) transduce a fall in glucose levels in the brain to release of NPY (Muroya et al., 1999). Increase in extracellular glucose levels inhibited NPY neurons of the ARC (Fioramonti et al., 2007). Interestingly, increased expression of NPY mRNA and protein was observed in the hypothalamus of diabetic rats, which was associated with the diabetes induced overeating (Ganguly, 2010; Zafar et al., 2014). In this background, NPY emerges as a critical factor linking diabetes with depression (Redrobe et al., 2005; Luo et al., 2008; Gupta et al., 2014), however, the possibility has not been tested experimentally.

This study was primarily aimed at evaluating the role of NPYergic system in the diabetes induced depression. Diabetic mice were injected with NPY, NPY Y1 receptor agonist [Leu^{31}, Pro^{34}]-NPY or antagonist BIBP3226 via the intracerebroventricular (icv) route, and screened for modulation of depression-like behavior using tail suspension test (TST). The antidepressant drug imipramine is known to increase the expression of NPY (Heilig et al., 1988), and its antidepressant effect is mediated by the NPY Y1 receptors (Goyal et al., 2009). Therefore, we examined the effects of imipramine on the profile of NPY expression in the extended amygdala inclusive of the central nucleus of amygdala (CeA), nucleus accumbens shell (AcbSh) and lateral division of the bed nucleus of stria terminalis (BNSTl) of the diabetic mice.

2. Results

2.1. Evaluation of the features of high fat diet (HFD)-fed streptozotocin (STZ) treated mice

Two weeks after HFD feeding, the mice showed significant increase ($p < 0.001$) in blood glucose levels as compared to that in control animals. One week after the STZ injection (3 weeks after HFD feeding) to HFD-fed mice, the blood glucose level was further increased drastically (more than 300 mg/dL), thus confirming the development of frank hyperglycemia i.e. T2D (Fig. 1(A)). In case of body weight measurements, animals on HFD for 2 weeks showed

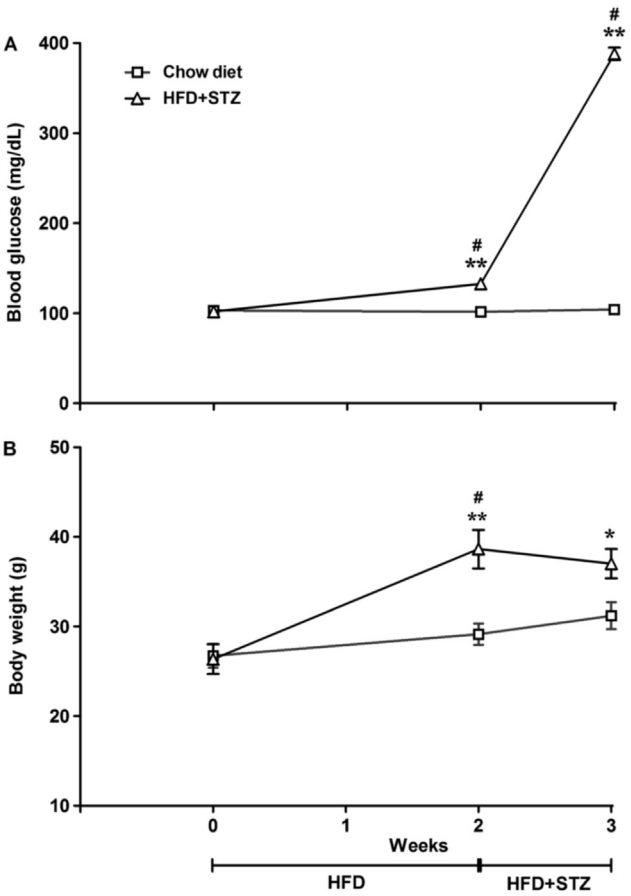

Fig. 1. Effect of high fat diet (HFD) and streptozotocin (STZ) treatment on blood glucose levels (A) and body weight (B). Different groups of mice were offered with HFD and chow diet. Two weeks after HFD feeding, STZ (35 mg/kg, ip) was injected. Non-fasting blood glucose level (mg/dL) and body weight (g) were monitored 2 weeks after dietary manipulations and 1 week after STZ injection. Blood glucose level and body weight of mice fed with chow diet were concomitantly measured. The data between the groups were analyzed by two-way ANOVA followed by Bonferroni's multiple comparison test. Moreover, the values of different time points within a group were analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test. Each line represents the mean ± S.E.M. of each group (n = 6 per group). $^{*}p < 0.05$,$^{**}p < 0.001$ vs chow diet; $^{\#}p < 0.001$ vs preceding time points.

significant increase in body weight ($p < 0.01$) as compared to that on chow diet. This weight gain was attenuated following administration of STZ (35 mg/kg, intraperitoneal; ip), although it was still considerably higher ($p < 0.05$) than body weight of chow fed group (Fig. 1(B)).

In oral glucose tolerance test, the high glucose levels were observed in the chow fed and HFD-fed mice at 30 min time point. However, in HFD-fed STZ treated mice, high glucose level was noticed at 30, 60 and 90 min time points (Fig. 2(A)). The area under the curve (AUC) of glucose in the HFD-fed STZ treated group was significantly bigger than that in the chow fed and HFD-fed groups ($p < 0.001$) (Fig. 2(B)). This delayed glucose disappearance confirms the development of insulin resistance i.e. T2D in HFD-fed STZ treated mice.

2.2. Effect of imipramine, NPY, [Leu^{31}, Pro^{34}]-NPY or BIBP3226 on immobility time in normal mice

Table 1 summarizes the dose-dependent effects of the treatments with imipramine (ip) and NPYergic agents (icv) on immobility time in non-diabetic mice. Treatment with imipramine at 15 mg/kg ($p < 0.05$) and 30 mg/kg ($p < 0.001$) doses significantly reduced immobility time as compared to that in saline treated