



ALTERED MET-ENKEPHALIN-LIKE PEPTIDES IN PLASMA OF GENETICALLY OBESE AND LEAN PIGLETS DURING DEVELOPMENT OF DIABETIC SYNDROME

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Opioids are involved into many physiological processes –also in the regulation of metabolism of carbohydrates, lipids and peptides. It is also known that native (free, pentapeptides) and cryptic (precursors) Met-enkephalin concentrations were changed in the pancreas of diabetic rats. The aim of the experiment was to estimate the changes of both forms of Met-enkephalin in the blood plasma of hyperglycemic piglets from two strains – wild, obese (Puławska) and lean, crossbreeds (PBZ). Piglets were divided into three groups: control (C), treated with streptozotocin (STZ) and treated with glucocorticoid (GLU). Plasma Met-enkephalin (native and cryptic) levels were measured by radioimmunoassay method. Plasma Met-enkephalin levels were significantly higher in Puławska piglets than in PBZ piglets -101.2±11.1 vs. 68.9±5.3 and 760.9±55.8 vs. 556.4±27.9 pmol/l , respectively for native and cryptic opioids. Induction of hyperglycemia by streptozotocin resulted in decreasing of both forms of enkephalin. However, the reaction of opioid system in obese piglets was stronger than in lean animals. In contrast, glucocorticoid injections increased the plasma glucose level similarly to STZ treatment but the opioid response was not so evident in PBZ piglets. Taking together, Met-enkephalin responses to hyperglycemia were different in obese and lean piglets.

Key words: opioids, hyperglycemia, piglets, metabolic syndrome

INTRODUCTION

Enkephalins, pentapeptides containing the consensus of Tyr-Gly-Gly-Phe-Xaa sequence, are the smallest of the molecules involved into modulation of many physiological processes. Opioids exist in small forms called native (free) peptides and in larger proteins termed cryptic (total) forms. Cryptic enkephalins are the source of native, acti-

ve pentapeptides which are released by enzymatic hydrolysis during stress, physical exercise or by stimulation with the hormones such as ACTH, glucocorticoids, catecholamines and insulin. Many experiments have shown that a decrease of native enkephalins in the blood is followed by the increase in its larger precursors (PIERZCHAŁA-KOZIEC and KEPYS, 2000, PIERZCHAŁA-KOZIEC et al., 2006).

Enkephalins are found in different parts of the brain and in some parts of the spinal cord that

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transmit pain impulses. In the spinal cord, enkephalins inhibit painful sensations by reacting with specific receptor sites on the sensory nerve endings. Enkephalins bind to opiate receptors and release controlled levels of pain. Met-enkephalin is involved in phenomena associated with modulated pain perception, regulation of memory and emotional conditions, food and liquid consumption and regulation of immunological system. It also has an impact on the digestive system motility, gastric as well as in pancreatic secretion and metabolism of carbohydrates (PIERZCHALA and VAN LOON, 1990).

The results of *in vivo* animal studies suggest that central administration of opiates and opioid peptides acts indirectly via the sympathetic nervous system to cause hyperglycemia and impaired insulin secretion, while peripheral administration tends to stimulate insulin and glucagon secretion (GIUGLIANO, 2001).

Opioid peptides stimulate intake of a high-fat diet more than a low-fat diet and in some studies preferentially increase the ingestion of pure fat more than carbohydrate, independent of baseline preference. A role for endogenous opioids in the feeding process is supported by evidence that feeding of a fat-rich diet and a preferential increase in fat intake are reduced by peripheral injection of opiate antagonists and also by central injection of selective antagonists.

Diabetes is a multifaced disease with different origins, and in spite of many tests, scientists are not able to find exact cause of the glucose metabolism disorders. Some results indicated that the obesity, genetic background or other disorders in the patient organism are responsible for diabetes induction. However, some of our results indicated that during stress, an excess of glucocorticoids might caused hyperglycemia in sheep, rats, hens and piglets (PIERZCHALA-KOZIEC et al.2010).

Opioids are also involved into modulation of metabolic syndrome, particularly diabetes, high triglycerides and cholesterol levels in piglets (PIERZCHALA-KOZIEC et al.2010). Piglets are a suitable biomedical model for energy metabolism and obesity in humans and to examine early changes in the neuronal functions connected with the beginning of metabolic syndrome (GERRITY et al., 2001).

In an effort to identify the early changes in the carbohydrates metabolism we have examined the levels of native and cryptic Met-enkephalin in diabetic piglets from obese and lean strains.

MATERIALS AND METHODS

The experiment was carried out on 36 piglets of two different strains – Polish Landrace (n=18), PBZ crossbreed, lean and Pulawska (n=18), wild, obese, at the age of 6 weeks and weight 12-14kg. Animals (females) were kept in Animal Station in standard conditions, fed with commercial food and were adapted to new environment for the period of 14 days before the experiment. Animals were divided into three groups (n=6) –control (C), treated with streptozotocin (STZ) and treated with glucocorticoids (GLU). Streptozotocin (STZ) was given i.p. three times every 2 days in a manner of 75, 50, 25 mg/ml of buffer (total of 150mg per piglet). Glucocorticoid was given i.p. three times, once a day at a total dose of 60 mg/kg b.w. Blood from ear vein was taken 24 hours after the last injection to heparinized tubes, immediately centrifuged for 30 minutes, 4000 rpm, at 4°C, plasma samples were stored at -70 °C until performing the radioimmunoassay of native and cryptic Met-enkephalin (PIERZCHALA and VAN LOON, 1990).

Briefly, native Met-enkephalin was extracted from plasma using Porapak chromatography columns, cryptic forms of Met-enkephalin were released from large peptides by enzymatic hydrolysis with trypsin and carboxypeptidase B, applied to Porapak columns and treated similarly to native opioid. Native enkephalin was purified on Porapak columns comprised of Porapak Q 100–120 mesh (Waters, Milford, Mass) in 3ml of absolute ethanol. Porapak slurry was prepared by degassing overnight 25 g of the material in 350ml of absolute ethanol. Shortly before applying the samples, columns were washed with 6ml of absolute ethanol and equilibrated with 9 ml of doubly distilled water. Columns loaded with the samples were washed with 6ml of doubly distilled water and native enkephalin was eluted with 3ml of absolute ethanol, then lyophilized and held at 4 °C until assay within 2 days. Met-enkephalin immunoreactivity was quantitated using commercial antiserum developed in rabbit (Bachem, Baden-Wuerttemberg, Germany), ¹²⁵I-Met-enkephalin (New England Nuclear, Boston, Mass) and Met-enkephalin standard (Peninsula, San Carlos, USA). The antiserum was used in a final dilution of 1:12 000; it showed cross-reactivity of 100% with Met-enkephalin sulfoxide-2% with Leu-enkephalin and less than 1% with Met-enkephalin-Arg-Phe, Met-enkephalin-Arg-Gly-Leu, or β -endorphin. Intra-as-

say and interassay coefficients of variation for the assay were 6 and 12 percent, respectively.

Results were expressed as means \pm SEM and their statistical comparison was made by analysis of variance (ANOVA) followed by Duncan's multiple range test.

All procedures were carried out in accordance with the Guide for Care and Use of Laboratory Animals and were approved by the Local Animal Care and Use Committee.

RESULTS

Native Met-enkephalin concentration (Fig. 1)

The concentration of native Met-enkephalin was 101.2 \pm 11.1 pmol/l in the plasma of control Pulawska and 68.9 \pm 5.3 pmol/l in PBZ piglets ($P<0.01$). Injections of streptozotocin significantly increased the plasma level of glucose (data not shown) and decreased the Met-enkephalin concentration to 18.7 \pm 3.3 pmol/l in obese and to 28.3 \pm 3.9 pmol/l in lean piglets ($P<0.01$). Injections of glucocorticoid also caused hyperglycemia and hyperinsulinemia (data not shown) and decreased the plasma level of native Met-enkephalin in obese piglets but only to the value of 82.2 \pm 4.9 pmol/l ($P<0.05$). Unexpectedly, in PBZ (lean) piglets glucocorticoids increased the plasma level of Met-enkephalin to 99.7 \pm 8.1 pmol/l ($P<0.05$).

Cryptic Met-enkephalin concentration (Fig. 2)

Streptozotocin decreased the concentration of cryptic Met-enkephalin from 760.9 \pm 55.8 (control obese animals) to 530.5 \pm 31.3 pmol/l ($P<0.01$). Decrease of endogenous precursor of enkephalin from 556.4 \pm 27.9 pmol/l to 474.7 \pm 18.3 pmol/l ($P<0.05$) was observed in the plasma of lean piglets after STZ treatment. Glucocorticoids augmented the decrease of cryptic Met-enkephalin in Pulawska piglets to 245.6 \pm 22.1 pmol/l and caused significant increase in PBZ piglets to 632.3 \pm 32.3 pmol/l ($P<0.01$).

Cryptic/native Met-enkephalin ratio (Fig. 3)

The ratio of cryptic to native peptide levels was increased by STZ from 7.5 \pm 0.9 in control Pu-

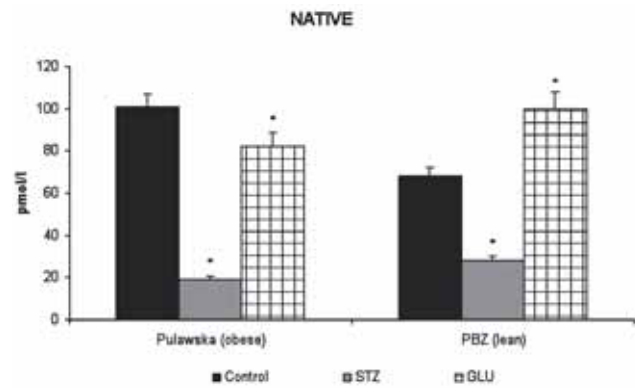


Fig. 1. The effect of STZ and glucocorticoids on the plasma native Met-enkephalin concentration in diabetic piglets (X \pm SEM, * $p<0.05-0.01$ compare to control values).

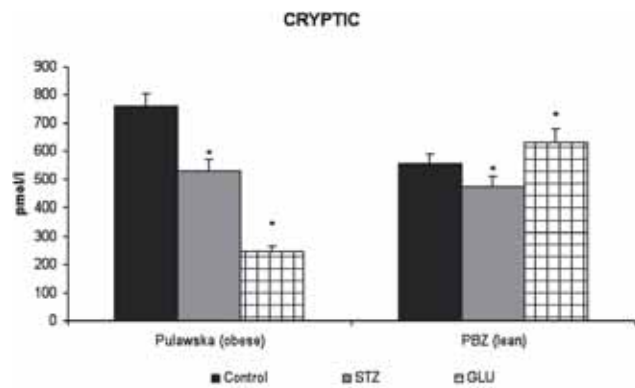


Fig. 2. The effect of STZ and glucocorticoids on the plasma cryptic Met-enkephalin concentration in diabetic piglets (X \pm SEM, * $p<0.05-0.01$ compare to control values).

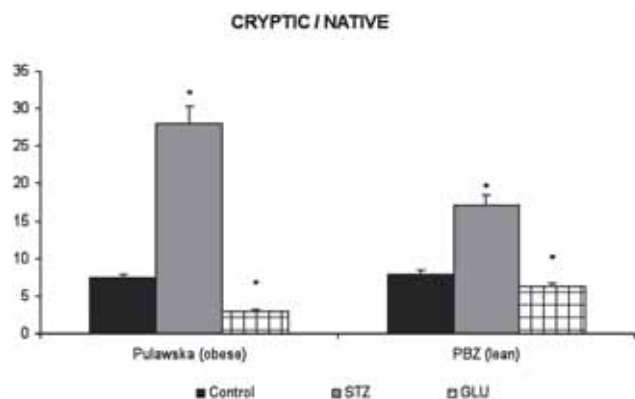


Fig. 3. The effect of STZ and glucocorticoids on the cryptic/native Met-enkephalin ratio in diabetic piglets (X \pm SEM, * $p<0.05-0.01$ compare to control values).

lawska and from 8.1 ± 0.8 in control PBZ animals to 28.4 ± 2.8 and 16.8 ± 1.4 , respectively ($p < 0.01$). Glucocorticoids decreased the ratio of cryptic to native Met-enkephalin in both strain of piglets ($p < 0.01$).

DISCUSSION

Our results clearly showed that diabetes in piglets was induced by both factors – streptozotocin which destroyed the islets of Langerhans (diabetes type 1) and glucocorticoids which affected the insulin receptors and caused insulin resistance (diabetes type 2). At the same time the plasma levels of glucose and insulin were very high but the levels of both forms of Met-enkephalin were significantly decreased. It must be pointed out that obese piglets (Pulawska strain) had higher levels of enkephalins and their reaction to STZ was stronger than in lean animals (PBZ strain). Also, the molecular ratio of cryptic to native Met-enkephalin in obese animals was 80% larger than in lean piglets. Probably, the synthesis and enzymatic hydrolysis of opioid precursor were inhibited by STZ acting as an inflammation agent. It seems reasonable, that native Met-enkephalin was involved into pain inhibition in animals treated with streptozotocin and that reaction to inflammation was stronger in wild type animals. In contrast, plasma Met-enkephalin levels (native and cryptic) were increased after glucocorticoids treatment only in lean animals. Wild type piglets had very low level of plasma cryptic and native enkephalins caused by glucocorticoids.

The question arises: whether induction of diabetes (by stress hormones -glucocorticoids) is determined by inherited tendency to obesity? Also, we must be aware that stress reaction is very complex and hypothalamo-pituitary-adrenal axis is always activated. It has been proposed that an unbalanced autonomic nervous system may be a major cause of the metabolic syndrome. Diabetes mellitus have also been reported to be accompanied by a number of behavioral and hormonal abnormalities, including hyperphagia, reduced motor activity. CNS abnormalities including neuronal atrophy and axonal degenerations are also associated with diabetes. The altered levels of neurotransmitter in specific brain areas in patients with diabetes mellitus and in animals with

experimental diabetes have been documented and implicated in the CNS disorders (RUDERMAN et al., 1992, EGLIN, 2002, GIREESH et al., 2009).

Numerous studies have demonstrated that diabetes stimulates chronic inflammation (KOH, 2000), so it is suggested that some of the peripheral and central nervous system pathophysiological symptoms associated with diabetes may be attributed, in part, to altered activity of enkephalinergic systems. Endogenous opioid peptides are involved into many different metabolic processes, also in the regulation of blood pressure and cardiovascular diseases as well as inflammation (PIERZCHAŁA-KOZIEC, 2010).

Our results showed decrease of the native and total Met-enkephalin levels in piglets with STZ-induced diabetes. Similar results were obtained by KOLTA et al., (1992,1996) in rats and it was connected with altered sensitivity of the dopaminergic receptors. That altered response of the dopaminergic system could be indirectly involved in the modulation of nociception in diabetic rats possibly through the enhancement and/or deactivation of the endogenous Met-enkephalinergic system.

Previous experiments showed that STZ-induced diabetes alters the enkephalinergic activity in some of these tissues. It is suggested that some of the peripheral pathophysiological symptoms associated with diabetes may be attributed, in part, to altered activity of enkephalinergic systems (KOLTA et al.,1992). Streptozotocine-induced diabetes alters the concentration of either or both forms of Met-enkephalin in plasma, the anterior and neurointermediate lobes of the pituitary, heart, lung, spleen, liver, seminal vesicle (KOLTA et al., 1996).

It can be possible that STZ caused decrease the synthesis of cryptic Met-enkephalin but increased its enzymatic processing to native forms, which are necessary for mediation of hypoalgesic response in diabetic piglets. This hypothesis is supported by the increasing the ratio of cryptic to native enkephalins. Previous experiments showed that long-term diabetes is associated with altered pain threshold and further support the hypothesis for endogenous opioid peptide mediation of hypoalgesia in chronically diabetic rats that can be prevented by insulin treatment (KOLTA et al.,1996).It is not clear, however, whether this difference in peptide immunoreactivity is related to a difference in peptide synthesis, storage or release, or to the

selective death of enkephalinergic neurons in the diseases (FALLUCA et al., 1996).

Thus our results revealed the significance of opioid peptides involvement in the diabetes regulation in piglets. These observations suggest that diabetes might be induced also by glucocorticoids excess in piglets and the severity of that disease is determined by inherited tendency to obesity.

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