

HYPERGLYCEMIA-INDUCED CHANGES IN RAT HIPPOCAMPAL MTOR AND OPIOID-LIKE PEPTIDES

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Hyperglycemia is listed as one of the major etiopathogenic factors for neurodegenerative disorders such as Alzheimer's disease (AD) which is associated with damage to the memory process. One of several kinases involved in many crucial processes in neurons and glial cells, like plasticity and memory formation, is mTOR. Changes in the activity of this kinase depending on the glucose status may affect different neural systems, e.g. the opioid-like peptide system. The present study was undertaken to examine the influence of hyperglycemia on the response of rat hippocampal neurons during mTOR inhibition. The experiment was carried out on adult male Wistar rats ($240.3g \pm 1.8g$, $n=40$). The animals were divided into 4 experimental groups: control, hyperglycemia, rapamycin – mTOR inhibitor (i.p. injections of 1 mg rapamycin/kg b.w. for three consecutive days) and hyperglycemia with rapamycin. Hyperglycemia was induced by a single i.p. injection of streptozotocin (STZ: 55 mg/kg b.w.). Twenty-four hours after the last injection of rapamycin the hippocampus was quickly removed. Tissue fragments were directed to *in vitro* incubation or homogenized, and Met-enkephalin concentration was measured in medium and the tissues by the RIA method. Also in homogenates the concentration of brain-derived neurotrophic factor (BDNF) was tested by ELISA. The obtained results showed that hyperglycemia modulated the opioid system function in the hippocampus and the observed response was correlated with mTOR activity.

Key words: hyperglycemia, opioids, hippocampus, BDNF, rapamycin

INTRODUCTION

Nowadays, metabolic changes associated with impaired glucose conditions are defined as diseases of civilization. Recent studies suggest that hyperglycemia may be the main factor leading to the development of neurodegeneration, e.g.

Alzheimer's disease which is called "Diabetes Type III" (DE LA MONTE and WANDS, 2008). It was observed that the main changes in neurones and glia cells were connected with mTOR kinase activity. Advanced research on the role of mTOR showed that inhibition of this signaling pathway had an essential impact on neuro-

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hormonal activity (MARTIN and HALL, 2005) Despite many efforts to determine the influence of the mTOR inhibitor on neural systems, its effect on endogenous opioid peptides has not been fully elucidated.

Opioids are endogenous peptides involved in modulating the activity of the nervous, endocrine and immune systems. The best known opioid peptides identified in mammals are endorphins, enkephalins and dynorphins (KONERU et al., 2009). Being present in both the central and peripheral nervous systems, opioids regulate many important reactions of the body, such as motivation, addiction, pain, stress responses and food intake. In addition to this well-established role, Met-enkephalin and its receptors also take part in modulation of cognitive functions (AL-HASANI and BRUCHAS, 2011; PRADHAN et al., 2012). High concentrations of Met-enkephalin were found in the striatum, hypothalamus and midbrain, in the limbic system, in the cerebral cortex, the pituitary gland and spinal cord (BODNAR, 2014). Met-enkephalin acts primarily through delta receptors but it may also bind to mu and zeta receptors (ZAGON et al., 1996). Mu and delta opioid receptors are widely distributed in the central nervous system, especially in the area of the hippocampus and hypothalamus and their activation modulates neuronal excitability at the level of the central (CNS) and peripheral (PNS) nervous systems (MONSOUR et al., 1994; POLAKIEWICZ et al., 1998).

The hippocampus is a critical structure for the consolidation of memory and learning processes. Studies of YOU et al. (2010) have shown that the use of morphine – mu and delta receptor agonist – resulted in activation of the PI3K / Akt-mTOR-p70S6K signaling pathway in neurons of the hippocampal CA3 field. The use of rapamycin and inhibitor of PI3K before administration of morphine inhibited the phosphorylation of Akt, mTOR and p70S6K. These results suggest that this pathway modulates cellular signal transduction induced by the response to morphine which acts through opioid receptors on the hippocampal area (YOU et al., 2010).

In the area of CNS endogenous opioids, such as Leu- and Met-enkephalin, through delta and mu receptors may also stimulate the expression of mRNA coding neurotrophic factors like BDNF.

Both the mTOR kinase pathway and the opioid system perform many important functions in

the body. The wide spectrum of action of mTOR and endogenous opioids might be essential for the functioning of the nervous system under pathological conditions, such as metabolic disorders, including hyperglycemia. Thus, the aim of the study was to investigate the influence of hyperglycemia on changes in the level of Met-enkephalin and neurotrophin under mTOR inhibition.

MATERIALS AND METHODS

The experiment was carried out on male Wistar rats ($240.3\text{g} \pm 1.8\text{g}$, $n=40$) housed under standard conditions ($22^\circ\text{C} \pm 0.5^\circ\text{C}$, 12L:12D) with free access to water and commercial feed. All the procedures were approved by the First Local Animal Ethics Commission in Krakow, Poland.

The animals were divided into 4 experimental groups: I – control, II – hyperglycemia (STZ), III – rapamycin (Rapa – mTOR inhibitor) and hyperglycemia with rapamycin (STZ+Rapa). First, in the groups II and IV, hyperglycemia was induced by a single i.p. injection of streptozotocin (STZ: 55 mg/kg b.w.). Rats were weighed prior to injection, and STZ was freshly dissolved in dilution buffer (0.1 M sodium citrate, pH 4.5, stored at 4°C). The animals in the groups I and III received a single i.p. injection of sterile saline. After 6 days, when hyperglycemia was stabilized, the animals in the groups III and IV received i.p. injections of $1\text{ mg rapamycin/kg b.w.}$ for three consecutive days. Twenty-four hours after the last injection of the mTOR inhibitor the animals were decapitated and the hippocampus was quickly removed. Tissues for determining the degree of Met-enkephalin secretion were placed in 24-well sterile plates containing 1 ml of Eagle's medium warmed to 37°C . After initial stabilization, final incubation of the tissues at 37°C in the medium was performed for 30 minutes. Tissue fragments for determination of Met-enkephalin and BDNF concentrations were homogenized in phosphate buffer (pH 6.5). Media and tissue fragments were stored at -80°C until analysis. Met-enkephalin concentration was estimated by radioimmunoassay (PIERZCHAŁA and VAN LOON, 1990) with chemicals from Peninsula Laboratories (USA). The BDNF level was measured by using the enzyme-linked immunosorbent commercial kit (Promega, USA), and protein concentration was estimated by the BCA commercial kit (Sigma-Aldrich, Poland).

The results were expressed as mean \pm SD and their statistical comparison was made by analysis of variance followed by Sheffe's test. Differences were considered significant at the level of 0.05 (Statistica 10, StatSoft Inc.,USA)

RESULTS

The obtained data showed that the single i.p. injection after 6 days caused hyperglycemia at the level of 18.59 ± 1.94 mM, while in the control group the glucose level was 5.07 ± 0.37 mM ($P < 0.05$).

The Met-enkephalin level in the hippocampus (Fig. 1) of the control rats was 61.69 ± 3.84 pmol/mg protein. Both hyperglycemia (STZ) and administration of rapamycin (Rapa) caused a significant ($P < 0.05$) increase in the Met-enkephalin concentration to 107.56 ± 17.39 pmol/mg protein and 132.46 ± 10.27 pmol/mg protein, respectively. The use of rapamycin in hyperglycemic animals (STZ + Rapa) reduced the opioid concentration by 55.22% (to the level of 48.16 ± 5.25 pmol/mg protein, $P < 0.05$).

The level of spontaneous Met-enkephalin secretion from the rat hippocampus (Fig. 2) was

decreased significantly only under the influence of hyperglycemia and rapamycin (STZ+Rapa) to the value of 97.90 ± 4.47 fmol/mg protein (a decrease by 67.06% compared to 297.19 ± 10.14 fmol/mg protein in the control group, $P < 0.05$).

The BDNF level in the hippocampus (Fig. 3) of the control animals was 32.21 ± 2.40 pg/mg protein. STZ-induced hyperglycemia did not cause significant changes but it was observed that the level of neurotrophin tended to increase (to 41.24 ± 7.88 pg/mg protein). The use of rapamycin did not affect significantly the BDNF level in the examined tissues regardless of the glucose status (Rapa: 36.33 ± 1.12 pg/mg protein; STZ+Rapa: 32.39 ± 3.19 pg/mg protein).

DISCUSSION

In recent years particular attention has been paid to the correlation between metabolic disorders and neurodegenerative diseases (MOSCONI et al., 2005; LEE et al. and 2011, BELTRÁN et al., 2012). The data presented above indicate that hyperglycemia may modulate opioid system activity by increasing the concentration of Met-enkephalin in the hippocampus. What is more, the similar

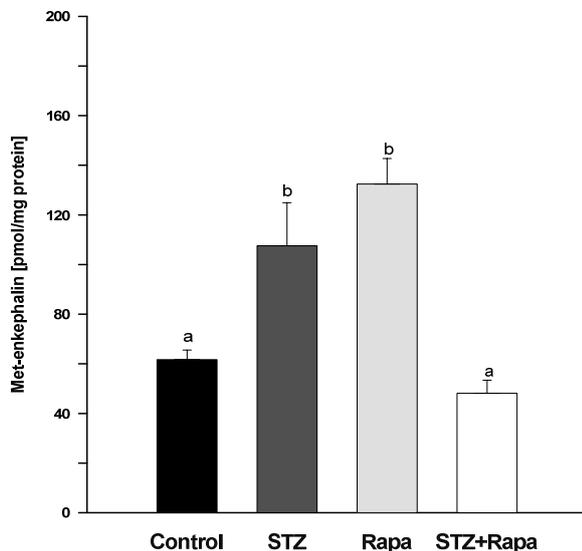


Fig. 1. Met-enkephalin concentration in the rat hippocampus ($X \pm SD$, $a, b P < 0.05$ – different superscript letters differ significantly). STZ – streptozotocin, Rapa – rapamycin.

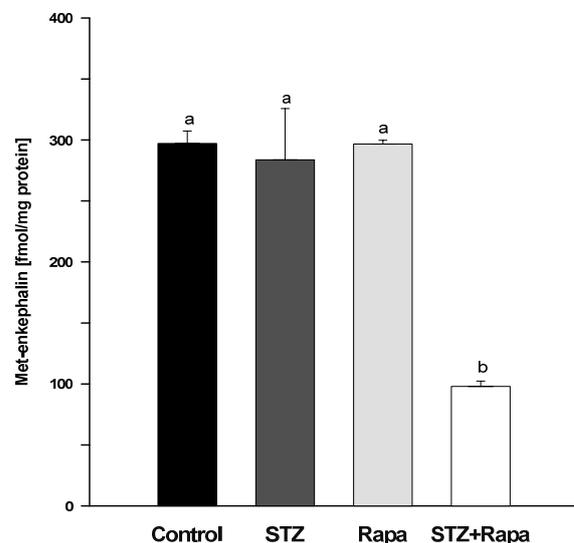


Fig. 2. In vitro Met-enkephalin secretion from the rat hippocampus ($X \pm SD$, $a, b P < 0.05$ – different superscript letters differ significantly). STZ – streptozotocin, Rapa – rapamycin.

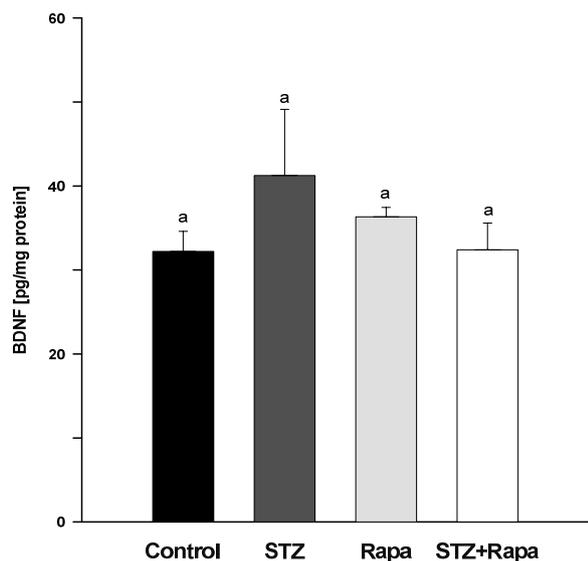


Fig. 3. BDNF concentration in the rat hippocampus ($X \pm SD$, ^{a,b} $P < 0.05$ – different superscript letters differ significantly). STZ – streptozotocin, Rapa – rapamycin.

response, i.e. the increased opioid level, was observed in animal tissues after mTOR kinase inhibitor treatment. In the rat hippocampus the arresting effect of the mTOR inhibitor on the opioid system was demonstrated only under impaired glucose conditions, which may also indicate a strong modulating influence of hyperglycemia on the mTOR pathway activity. The results of one of the recent studies carried out on a line of rat cortical neurons also confirmed the existence of interactions between the opioid system and the mTOR pathway in the CNS. Morphine inhibited the neurotoxic effect of A β oligomers on cortical neurons and induced increased activity of the mTORC1 pathway by stimulating PI3K. The observed reactions indicated the potential use of opioids for inhibition of the development of atherosclerotic changes and neurodegenerative diseases by modulation of the mTOR signalling pathway (WANG et al., 2015). The results obtained for *in vitro* Met-enkephalin secretion from the rat hippocampus additionally confirmed the existence of interactions between the opioid system and the mTOR pathway. This relationship was noted under hyperglycemic conditions with rapamycin treatment in which a reduction in the opioid level was observed.

In mature animals BDNF is involved in the process of synaptic plasticity based on long-term

potentiation. The results of clinical observation of patients suffering from neurodegenerative disorders, schizophrenia or depression suggested that the measurement of BDNF in the blood plasma may be a useful biomarker for CNS pathology. The data obtained from experiments on animals showed that the peripheral neurotrophin level was positively correlated with the level of hippocampal BDNF in pigs and rats (KLEIN et al., 2011). Although in the present study in all the experimental groups there were no significant changes in the concentration of BDNF, the neurotrophin level tended to increase under hyperglycemia. This reaction may indicate that the neuroprotective reaction started under impaired glucose conditions, and the reaction could be mediated by an increase in the Met-enkephalin concentration. Earlier studies by ZHANG et al. (2006) confirmed that the effect of enkephalins on BDNF mRNA expression in the hippocampus occurred through delta and mu receptor-mediated mechanisms (ZHANG et al., 2006).

Cognitive impairment, characteristic of neurodegeneration, was also documented in individuals in which hyperglycemia led to changes in the brain functions (GLASS et al., 2010; SELVARAJAH et al., 2013). The present study additionally highlights that hyperglycemia influences the opioid system, e.g. by increasing the level of Met-enkephalin in the hippocampus, which may result in higher concentrations of neuroprotective factors.

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