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Tramadol enhances hepatic insulin sensitivity via enhancing insulin signaling cascade in the cerebral cortex and hypothalamus of 90% pancreatectomized rats

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Abstract

Clinical observation found that tramadol, mu opioid receptor (MOR) agonist and serotonin (5-HT) reuptake inhibitor, has a hypoglycemic effect in type 2 diabetes patients. The mechanism of its hypoglycemic effect has not been fully defined. This study showed that tramadol activated a neuronal insulin signaling cascade by increasing the induction of insulin receptor substrate-2 expression in primary cultured neuronal cells while this activation was suppressed by naloxone (MOR inhibitor) and dexamethasone (non-specific inhibitor of MOR and 5-HT receptor, DEX). Glucose utilization of the cerebral cortex and hypothalamus was enhanced by a 4-week-tramadol administration in 90% pancreatectomized rats, in vivo, as assessed by measurement of glucokinase expression and glycogen deposition via activating insulin signaling cascade such as neuronal cells in vitro. This improvement was almost completely suppressed by naloxone as well as DEX. Tramadol decreased fasted serum glucose levels, favored an increase in the glucose infusion rate and reduced endogeneous hepatic glucose production after 4 weeks of treatment. However, tramadol did not modulate hepatic glucose output directly, as exhibited by liver perfusion, suggesting tramadol altered hepatic glucose utilization through the effect of organs other than the liver, possibly the central nervous system. The data suggest that tramadol ameliorates peripheral glucose metabolism through central activation of MOR, and that central and peripheral glucose metabolism are therefore likely to be interrelated.

Keywords: Dexamethasone; Mu receptor agonist; Serotonin reuptake inhibitor; Cerebral cortex; Hypothalamus; Neuronal cells

1. Introduction

Our clinical observations revealed distinctive phenomena in type 2 diabetic patients who began continuous subcutaneous insulin infusion therapy (CSII) via insulin pump (Dana Diabecare II, Sooil, Korea). At the beginning of CSII therapy, most patients' blood glucose levels normalized within 1–2 weeks during which they exhibited eating disorders, cortisol insufficiency and severe pain sensations characteristic of diabetic peripheral neuropathy. The symptoms disappeared with daily treatments of 1 mg dexamethasone (DEX) and 40 mg tramadol, a centrally acting mu opioid receptor (MOR) agonist and serotonin (5-HT) reuptake inhibitor. Two to three months later, omitting tramadol increased blood glucose levels by approximately 3.3–4.4 mM without any reported change in pain sensation. Re-administration of tramadol made blood glucose levels return to normal without changing the insulin dosage in the patients (unpublished data). In other words, tramadol demonstrated hypoglycemic effects in type 2 diabetes patients.

Abbreviations: CREB, cAMP response element binding protein; CSII, continuous subcutaneous insulin infusion therapy; DEX, dexamethasone; DMEM, Dulbecco's Modified Eagle Medium; 5-HT, serotonin; HPA, hypothalamus-pituitary-adrenal glands; IRS, insulin receptor substrate; MOR, mu opioid receptor; PKB, protein kinase B

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Ligands of the MOR, including morphine and β-endorphin, are known to have profound effects on peripheral metabolism such as blood pressure, heart rate and respiration [16]. While some of these effects may be mediated by receptors located on peripheral tissues [4] or vagal afferents [34], central opioid receptors are also important [26]. Although MOR was widely distributed within the central nervous system (CNS) and peripheral tissues, specific region and content variation existed [4,35]. According to the detection techniques, MOR expression was reported to be varied. MOR was expressed mainly in the cerebral cortex and hypothalamus within CNS, and in the small intestine, kidney, adrenal, heart and spleen within peripherals [4,35]. However, MOR was minimally expressed in hepatocytes.

Tramadol, a 4-phenylpiperidine analogue of codeine, has been developed as an atypical centrally-acting opioid agent [29]. It activates the opiatergic and the serotonergic systems in the CNS [3,29] to reduce peripheral pain. With its unique dual mechanisms of action, tramadol provides excellent analgesia, particularly in the treatment of moderate to moderately severe pain [36]. Thus, tramadol works through the CNS to reach the peripherals. In contrast to other opioids, the analgesic action of tramadol is only partially inhibited by an MOR antagonist, naloxone, which suggests the existence of another mechanism of action.

DEX mediates various metabolisms through the CNS and peripheries. One of them is glucose homeostasis. DEX has been known to induce hyperglycemia, hyperinsulinemia and insulin resistance through the suppression of glucose utilization directly in liver and adipose tissues and indirectly via the CNS [2]. Some studies have provided evidences that DEX influences brain glucose metabolism and indirectly modulates peripheral glucose metabolism in cell culture and animal studies [1,13,18,28]. Exposure of cortical astrocytes to DEX resulted in the reduction of noradrenaline-induced glycogen synthesis in a concentration-dependent manner [1]. DEX increased peripheral insulin resistance in vivo, which was connected to the suppression of the hypothalamus-pituitary-adrenal glands (HPA) axis [18]. In addition, DEX selectively suppressed the MOR and 5-HT receptors, especially the 5-HT_{1A} receptor in the brain [13,28]. Thus, DEX was selected as a broad inhibitor of tramadol in this study, and naloxone was used as a specific inhibitor of MOR in order to determine whether tramadol can improve glucose homeostasis through MOR and/or 5-HT receptors.

The long-term effect of tramadol on glucose metabolism and insulin signaling cascade was determined in the CNS and periphery of 90% pancreatectomized (Px) male Sprague–Dawley rats. The changes of peripheral glucose metabolism were examined by measuring insulin sensitivity and insulin secretion capacity. Px rats were a good model in which to study this effect since they have moderate symptoms of type 2 diabetes, especially Asian diabetes, a combination of insulin resistance and insulin insufficiency.

2. Materials and methods

2.1. Primary neuronal cell culture

Primary neuronal cell cultures were prepared from dissociated forebrain (mostly cerebral cortex) neurons prepared from the brains of embryonic Sprague–Dawley rat fetuses at 18 gestational days [10]. The uteri of the pregnant rats were removed under sterile conditions. After the embryonic brains were removed, the neocortices were digested with 0.25% trypsin (Invitrogen, Carlsbad, CA) and 0.1% deoxyribonuclease (Sigma, St. Louis, MO) for 15 min at 37 °C and separated into a single-cell suspension by mechanical titration through Pasteur pipettes. Cells were then plated in 35 mm dishes previously coated with 100 μg/mL poly-L-lysine (Sigma) in a density of 1×10^6 cell per dish. The culture medium consisted of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) with high glucose containing 10% heat inactivated fetal calf serum (Invitrogen) and 10% heat inactivated horse serum (Invitrogen); 50 IU/mL penicillin and 50 μg/mL streptomycin; 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma); 2 mM glutamine. After 20 h of incubation, the proliferation of non-neuronal cells was inhibited by the addition of 10 μM cytosine arabinoside. The cultures were used 9–12 days after plating the cells. After a pre-incubation of 5 μM DEX or 5 μM naloxone (Sigma) for 20 min, cells were treated with 20 μM tramadol in high glucose DMEM with 10% serum for the designated periods. Immediately before the designated period ended, 100 nM insulin was administered for 10 min and the cells were harvested in ice-cold PBS and lysed in the previously described lysis buffer. After 30 min of incubation on ice, the lysates were centrifuged for 10 min at 12,000 rpm at 4 °C.

2.2. Animals

Male Sprague–Dawley rats weighing 251.1 ± 15.6 g (10 weeks old) were anesthetized with an intraperitoneal injection of ketamin and xylazin (100 mg and 10 mg/kg body weight). Using the Hosokawa technique [17], 90% of each pancreas was removed. The remnant (residual pancreas) was anatomically well defined, being the tissue within 2 mm of the common bile duct and extending from the duct to the first part of the duodenum. Within the group of Px rats, those with randomly fed serum glucose levels less than 9.4 mM were eliminated after 2 weeks of surgery. They were housed one to a cage at 22–23 °C with a 12-h light:12-h dark cycle. The animals were allowed free access to standard laboratory food (Sam Yang Co, Kangwon-Do, Korea) and standard tap water. The Konkuk University Animal Care and Use Committee approved all procedures performed in this study.

2.3. In situ liver perfusion

Two weeks after the 90% pancreatectomy, in situ liver perfusion was performed in fasting rats to determine whether