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# Acute stimulation of brain mu opioid receptors inhibits glucose-stimulated insulin secretion via sympathetic innervation

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ABSTRACT

Pancreatic insulin-secreting β-cells express opioid receptors, whose activation by opioid peptides modulates hormone secretion. Opioid receptors are also expressed in multiple brain regions including the hypothalamus, where they play a role in feeding behavior and energy homeostasis, but their potential role in central regulation of glucose metabolism is unknown. Here, we investigate whether central opioid receptors participate in the regulation of insulin secretion and glucose homeostasis *in vivo*. C57BL/6J mice were acutely treated by intracerebroventricular (i.c.v.) injection with specific agonists for the three main opioid receptors, kappa (KOR), delta (DOR) and mu (MOR) opioid receptors: activation of KOR and DOR did not alter glucose tolerance, whereas activation of brain MOR with the specific agonist DAMGO blunted glucose-stimulated insulin secretion (GSIS), reduced insulin sensitivity, increased the expression of gluconeogenic genes in the liver and, consequently, impaired glucose tolerance. Pharmacological blockade of $\alpha_{2A}$-adrenergic receptors prevented DAMGO-induced glucose intolerance and gluconeogenesis. Accordingly, DAMGO failed to inhibit GSIS and to impair glucose tolerance in $\alpha_{2A}$-adrenoceptor knockout mice, indicating that the effects of central MOR activation on β-cells are mediated via sympathetic innervation. Our results show for the first time a new role of the central opioid system, specifically the MOR, in the regulation of insulin secretion and glucose metabolism.


## 1. Introduction

The opioid system is mostly known for its pain-relieving and addictive properties, yet it also regulates other processes in the body, such as appetite and energy expenditure, (Bodnar, 2016; Nogueiras et al., 2012). There are three well-known opioid receptors, delta (δ-, DOR), kappa (κ-, KOR) and mu (μ-, MOR), all of them belonging to the G coupled-protein receptors family (Al-Hasani and Bruchas, 2011). Their natural ligands are endorphins, enkephalins, and dynorphins, which derive from different precursor proteins: proopiomelanocortin (POMC), preproenkephalin (PENK) and preprodynorphin (PDY), respectively (Koneru et al., 2009). In mammals, both opioid peptides and opioid receptors are widely expressed in many tissues. Within the brain, MOR and KOR are highly expressed in thalamus, hypothalamus, cerebral cortex, striatum, cerebellum and certain areas of the brainstem and spinal cord, while DOR is primarily expressed in the cerebral cortex (Peckys and Landwehrmeyer, 1999). Cells expressing POMC are mainly located in the arcuate nucleus of the hypothalamus (ARC), the nucleus tractus solitarius (NTS) and areas of the pituitary, whereas cells expressing PENK or PDY are widespread in multiple regions of the brain (Le Merrer et al., 2009).

Signaling through opioid receptors on the endocrine pancreas

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modulates insulin secretion from β-cells. Reportedly, β-endorphin and high doses of enkephalin analogues inhibit insulin release (Garcia-Barrado et al., 2002; Green et al., 1980; Schleicher, 1989), whereas dynorphin, low doses of enkephalin and morphine stimulate insulin secretion *in vitro* (Green et al., 1980, 1983). In contrast, inhibition of glucose-stimulated insulin secretion (GSIS) by dynorphin and morphine was observed in studies carried out in rat perfused pancreas (Ishizuka et al., 1986). The intravenous administration of β-endorphin has been reported to inhibit insulin secretion (Fatouros et al., 1997; Giugliano et al., 1989), although its effects on insulin release seem to be dependent on the blood glucose levels (Khawaja and Green, 1991). In addition, knockout (KO) mice lacking MOR (Wen et al., 2009) or DOR (Czyzyk et al., 2012), but not KOR (Czyzyk et al., 2010), display improved glucose tolerance, accompanied by an enhanced insulin secretion upon glucose exposure both *in vivo* and *in vitro* (Wen et al., 2009). Taken together, these findings have led to the general view that insulin secretion is regulated by peripheral islet opioid receptors. Prompted from recent findings indicating an important role of the CNS in glucose homeostasis, here we investigate the effects of acute and chronic activation of hypothalamic opioid receptors on insulin secretion and glucose tolerance. Our data shows for the first time a new role of the central opioid system, specifically the MOR, in the regulation of glucose homeostasis through a mechanism involving insulin secretion, reduced insulin sensitivity and increased hepatic glucose production.

## 2. Materials and methods

### 2.1. Animals

8-10-weeks old C57BL/6J male mice (Animalario General of USC; Santiago de Compostela, Spain), and 6 months old $\alpha_{2A}$-adrenergic receptors KO mice ($\alpha_{2A}$-KO) and wild type (WT) mice, both on FVB-background (central animal research facility (ZETT), Heinrich-Heine University, Düsseldorf, Germany), were kept under a 12 h light/dark cycle and had *ad libitum* access to chow diet and water. For the studies with mice fed a HFD, 6-week-old C57BL/6J mice were switched from a regular chow to HFD D12492 (60% kcal from fat, 20% kcal from carbohydrates and 20% kcal from protein; Research Diets, New Brunswick, NJ) for 3 months before undergoing the studies. All experimental procedures were reviewed and approved by the Ethics Committees of the University of Santiago de Compostela, in accordance with the institutional guidelines and in strict compliance with the European Union normative for the care and use of experimental animals.

### 2.2. Intracerebroventricular (i.c.v.) infusion

For all surgical procedures, animals were placed under anesthesia by intraperitoneal (i.p.) injection of ketamine/xylazine (140 and 7 mg/kg body weight, respectively), and administered ketoprofen (5 mg/kg body weight) and 250 μl of saline by subcutaneous injection. For acute administration, a cannula was positioned on the right lateral cerebral ventricle as previously described (Nogueiras et al., 2009). For chronic infusion, a Brain Infusion Kit 3 (ALZET; DURECT, USA) was fixed on the lateral ventricle, and an Alzet 7-day pump (ALZET; DURECT, USA) connected to the kit was implanted subcutaneously. After surgery, animals were housed individually, and daily body weight and food intake measurements were obtained in the morning for the chronic studies. [D-Ala2, NMe-Phe4, Gly-ol5]-enkephalin (DAMGO), trans-(-)-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide hydrochloride (U-50.488), (+)-4-[(α*R*)-α-((2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC-80) and β-Funaltrexamine hydrochloride (β-FNA) were purchased from Tocris, USA (stocks were dissolved in distilled $H_2O$ or DMSO, according to manufacturer's instructions, and brought to experimental doses by dissolving in saline). The final volume of each i.c.v. injection was 2 μl.

### 2.3. Glucose tolerance test and glucose-stimulated insulin secretion (GSIS)

Mice were fasted for 6 h and then given an i.p. glucose load (2 g/kg body weight) of a 20% glucose solution (Tuduri et al., 2015). Blood was sampled from the tail vein (for glucose tolerance test) or from the saphenous vein (for GSIS). In both procedures, blood glucose was measured before glucose injection (0 min) and at different time points, using a Glucocard glucometer (A. Menarini diagnostics, Spain). For GSIS, blood was also measured for insulin at different time points by an Insulin Mouse enzyme-linked immunosorbent assay (ELISA) (Millipore, USA). The areas under the curve (AUC) were calculated from 0 to 120 min (for glucose) and from 0 to 60 min (for insulin) and baselines were set at the fasting levels.

### 2.4. Intraperitoneal (i.p.) injections

For acute i.p. injections, DAMGO (0.4 nmol) or RS79948 (1 mg/kg body weight, Tocris Bioscience, Bristol, UK) were injected in a final volume of approximately 200 μl.

### 2.5. Insulin tolerance test

Mice were fasted for 6 h and then given an i.p. injection of insulin (Actrapid) at a dose of 0.65 U/kg of body weight. Blood glucose was measured from the tail vein at the time points indicated in the figure.

### 2.6. Streptozotocin (STZ) and nicotinamide (NA) treatment

Mice received nicotinamide (1000 mg/kg body weight, 72340 Sigma Aldrich, USA) and streptozotocin (150 mg/kg body weight, S0130 Sigma Aldrich, USA) by i.p. injection as previously described (Tahara et al., 2011).

### 2.7. Plasma glucagon measurements

Plasma glucagon levels were measured by means of a mouse glucagon ELISA-10 μl kit (Mercodia, Sweden).

### 2.8. Western blot analysis

Total protein lysates from hypothalamus (16 μg), were subjected to SDS-PAGE, electrotransferred onto a polyvinylidene difluoride membrane and probed with Anti-MOR antibody (ab17934, dilution 1:1000) (Abcam, UK) and monoclonal Anti-α-tubulin antibody (T5168, dilution 1:5000) (Sigma Aldrich, USA). For protein detection we used horseradish peroxidase-conjugated secondary antibodies and chemiluminescence (Amersham Biosciences, UK). Afterwards, membranes were exposed to radiograph film (Super RX Fuji Medical X-Ray Film; Fujifilm, Japan) and developed with developer and fixing liquids (AGFA, Belgium) under appropriate dark room conditions. Protein expression was quantified by densitometric analysis with ImageJ software (http://rsbweb.nih.gov/ij/). Protein levels were normalized to α-tubulin.