Brief Communication

MOLECULAR METABOLISM

Galanin enhances systemic glucose metabolism through enteric Nitric Oxide Synthase-expressed neurons

Anne Abot [1,2], Alexandre Lucas [3], Tereza Bautzova [1,2], Arnaud Bessac [1,2], Audren Fournel [1,2], Sophie Le-Gonidec [3], Philippe Valet [3], Cédric Moro [3], Patrice D. Cani [2,4,], Claude Knauf [1,2,*]**

ABSTRACT

Objective: Decreasing duodenal contraction is now considered as a major focus for the treatment of type 2 diabetes. Therefore, identifying bioactive molecules able to target the enteric nervous system, which controls the motility of intestinal smooth muscle cells, represents a new therapeutic avenue. For this reason, we chose to study the impact of oral galanin on this system in diabetic mice.

Methods: Enteric neurotransmission, duodenal contraction, glucose absorption, modification of gut—brain axis, and glucose metabolism (glucose tolerance, insulinemia, glucose entry in tissue, hepatic glucose metabolism) were assessed.

Results: We show that galanin, a neuropeptide expressed in the small intestine, decreases duodenal contraction by stimulating nitric oxide release from enteric neurons. This is associated with modification of hypothalamic nitric oxide release that favors glucose uptake in metabolic tissues such as skeletal muscle, liver, and adipose tissue. Oral chronic gavage with galanin in diabetic mice increases insulin sensitivity, which is associated with an improvement of several metabolic parameters such as glucose tolerance, fasting blood glucose, and insulin.

Conclusion: Here, we demonstrate that oral galanin administration improves glucose homeostasis *via* the enteric nervous system and could be considered a therapeutic potential for the treatment of T2D.

Keywords Galanin; Enteric nervous system; Diabetes

1. INTRODUCTION

Inter-organ communication is of crucial importance for the maintenance of glucose homeostasis. Inter-organ communication comprises hormonal and nervous regulation, including the hypothalamus, playing a major role as a center of integration of various nervous, and peripheral signals, including nutrients and afferent signal from the intestine [1,2]. Thus, the modification of hypothalamic neuronal activity *via* the gut induces large variations of peripheral glucose utilization in organs such as muscles, liver, and white adipose tissues [3]. Hence, it is clear that new therapeutic strategies for type 2 diabetes (T2D) should take into consideration the gut-to-brain axis as major primary site of regulation of glucose homeostasis [4].

Recently, a new concept has emerged demonstrating that the enteric nervous system (ENS) could be targeted by bioactive factors to control glucose utilization in skeletal muscle *via* a hypothalamic relay [5]. The ENS is mostly composed of Choline Acetyl Transferase (ChAT) and neuronal Nitric Oxide Synthase (nNOS) neurons that respectively stimulate or inhibit intestinal smooth muscle cells. In fact, the ENS alteration observed in proximal intestine of obese/diabetic mice and human generates duodenal hyper-contractility in the fed state [6]. Thus, this aberrant duodenal hyper-contractility first leads to an increase of glucose absorption that contributes to chronic hyperglycemia observed during T2D and then to the genesis of an afferent message from the gut to the hypothalamus that results in an insulin resistant state [5]. Therefore, discovering new molecular actors that able to modulate ENS activity in order to decrease duodenal hyper-contractility represents an original therapeutic approach to improve T2D [7]. Among all potential signaling factors, bioactive peptides could be considered preferential therapeutic targets. In this context, numerous bioactive peptides (e.g., leptin, apelin) present in the intestinal lumen can reach the myenteric plexus to modulate the activity of ENS neurons [5,8].

[1]Institut National de la Santé et de la Recherche Médicale (INSERM), U1220, Université Paul Sabatier, UPS, Institut de Recherche en Santé Digestive et Nutrition (IRSD), CHU Purpan, Place du Docteur Baylac, CS 60039, 31024 Toulouse Cedex 3, France [2]NeuroMicrobiota, European Associated Laboratory (EAL) INSERM/UCL, France [3]Institut National de la Santé et de la Recherche Médicale (INSERM), U1048, Université Paul Sabatier, UPS, Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), CHU Rangueil, 1 Avenue Jean Poulhès, BP84225, 31432 Toulouse Cedex 4, France [4]Université Catholique de Louvain (UCL), Louvain Drug Research Institute, LDRI, Metabolism and Nutrition Research Group, WELBIO (Walloon Excellence in Life sciences and BIOtechnology), Avenue E. Mounier, 73 B1.73.11, B-1200, Brussels, Belgium

*Corresponding author. Institut National de la Santé et de la Recherche Médicale (INSERM), U1220, Université Paul Sabatier, UPS, Institut de Recherche en Santé Digestive et Nutrition (IRSD), CHU Purpan, Place du Docteur Baylac, CS 60039, 31024 Toulouse Cedex 3, France. E-mail: claude.knauf@inserm.fr (C. Knauf).

**Corresponding author. Université catholique de Louvain (UCL), Louvain Drug Research Institute, LDRI, Metabolism and Nutrition Research Group, WELBIO (Walloon Excellence in Life sciences and BIOtechnology), Av. E. Mounier, 73 B1.73.11, B-1200, Brussels, Belgium. E-mail: patrice.cani@uclouvain.be (P.D. Cani).

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Galanin is a neuropeptide largely expressed in the brain but also in ENS neurons [9]. Galanin is known to have beneficial effects on glucose metabolism *via* central and peripheral actions [10,11]. So far, three galanin receptors have been identified (Gal-R1, Gal-R2, and Gal-R3), and their expression differs according to the cell type in which they are expressed and the development state [12]. In the intestine, the majority of galanin effects are mediated by Gal-R1 predominantly expressed in ENS neurons [13] and more particular in neurons that express acetylcholine, nNOS, or vasoactive intestinal peptide [14]. More precisely, galanin is known to be a neuropeptide that exerts an inhibitory action on myenteric cholinergic neurons [15]. Galanin is also expressed in enteric neurons, which receive inhibitory and stimulatory information from noradrenergic and cholinergic nerves fibers respectively [16].

During T2D, the expression of galanin is decreased in the duodenum of mice [17], thereby suggesting a potential benefic impact of this peptide on gut-to-brain axis and the control of glucose metabolism. This hypothesis is supported by data showing that galanin inhibits cholinergic transmission in the ENS to favor the relaxation of intestinal smooth muscle cells [18] and that Gal-R1 knockout mice have a significant increase in glycemia in the fed state [19].

Whether galanin is able to modulate the ENS/duodenal contractions to improve glucose homeostasis remains to be determined. Here, our main objective is to unravel the effect of galanin on nitric oxide (NO) release from enteric nNOS neurons, which represent the major population of inhibitory neurons of intestinal contraction, and to evaluate the consequence of this intestinal effect of galanin on gut-to-hypothalamus axis in the control of glucose metabolism.

2. MATERIALS AND METHODS

2.1. Mice

Nine-week-old male C57BL/6J mice (Charles River Laboratory, l'Arbresle, France) were housed in specific pathogen free conditions and in a controlled environment (room temperature of 23 ± 2 °C, 12 h daylight cycle) with free access to food and water. Experiments were conducted according to the European Community regulations concerning the protection of experimental animals and were approved by the local Animal Care and Use Committee. Mice were fed on normal chow (NC) diet or high-fat diet (HFD) containing 20% protein, 35% carbohydrate, and 45% fat (Research Diet, New Brunswick, NJ, USA).

2.2. Isotonic contraction

Mice were euthanized in fed conditions. After dissection, duodenum segments were washed in Krebs–Ringer bicarbonate/glucose buffer (pH 7.4) in 95% O_2 and 5% CO_2. Segments of duodenum were then incubated in oxygenated Krebs–Ringer solution for 30 min at 37 °C, attached to the isotonic transducer (Lever Transducer, B40 type 373, Hugo Sachs Elektronik, Grünstraße, Germany), and immersed in Falcon tubes containing 25 mL of the same medium maintained at 37 °C. The load applied to the lever was 2g (20 mN). Isotonic contractions were recorded on BDAS software (Hugo Sachs Elektronik) following the transducer displacement. After attaching the duodenum segments, basal contractions were recorded for 10 min. Subsequently, 100 µL of Krebs–Ringer solution or specific drugs (Galanin 100 nM ± galantide 100 nM) were added to the survival medium, and contractions were recorded for 10 min. Amplitudes were recorded for 10 min at 10-second intervals and the average was compared to the average basal contractions. Contraction amplitudes are presented as percentage relative to the basal response whilst contraction frequencies are presented as number of contraction per minute as previously described [5]. For the one-week chronic oral galanin gavage protocol, basal contractions were recorded for 5 min and are presented as average basal contractions. Here, the mice were treated daily with an oral gavage of 100 µL of each drug (Control or galanin 100 nM) during the last week of HFD treatment as previously used [5]. These mice became obese and insulin resistant after 3 months of HFD [5]. To evaluate duodenal contractility, data have been recorded in fed mice, corresponding to the phase in which intestinal segmental waves are generated to increase the rate of nutrients absorption [5,20]. Data representation in % of basal contraction refers to a basal value obtained before the actual experiment.

2.3. Telemetry

Under anesthesia with isoflurane, a needle attached to a catheter was placed in the stomach for intragastric injection of H_2O or galanin. Two electrodes were sutured 5 mm from each other, on the proximal duodenum, 1.5 cm caudal to the pylorus. The signal, corresponding to the electrical activity of duodenum, was received by an RMC-1 receiver (DSI) placed under the cage. The signal obtained during 40 min of continuous recording was analyzed off-line to obtain the integral of the rectified signal over the 100 ms integration interval. The electric signal was allowed to stabilize during 5 min [5]. The next 5 min were considered as basal signal, and then the mice received H_2O or drugs (galanin 100 nM ± galantide 100 nM) by intragastric injection of 100 µl at a rate of 10 µl/min using a syringe pump. Data representation in % of basal electrical signal refers to a basal value obtained before the actual experiment.

2.4. Intestinal NO real-time measurement

Mice were euthanized in fed conditions. After dissection, duodenum fragments were washed in Krebs–Ringer bicarbonate/glucose buffer (pH 7.4) in 95% O_2 and 5% CO_2 and then immersed in Eppendorf tubes containing 400 µL of the same medium. After a 10 min recovery period, the spontaneous NO release was measured at 37 °C for 5 min by using a NO-specific amperometric probe (ISO-NOPF, 100 µm diameter, 5 mm length, World Precision Instruments, Aston Stevenage, UK) implanted directly in the duodenum and in response to each drugs (Control, galanin 100 nM ± galantide 100 nM) [5].

2.5. Hypothalamic NO real-time measurement

Mice in fed conditions were anesthetized with isoflurane. A 1-cm midline incision was made across the top of the skull, and the animal was placed on a stereotaxic apparatus, as described previously [5]. During amperometric measurement, animals received H_2O or drugs *via* an intragastric injection of 10 µl/min rate for 10 min, using a syringe pump. We previously demonstrated that H_2O is the vehicle of choice for glucose administration [1,21]. Furthermore, we have also demonstrated that intragastric perfusion of H_2O did not modify c-Fos expression (a marker of neuronal activity) in the hypothalamus, as compared with NaCl [21]. Therefore, all control groups were infused with H_2O, as previously described in detail [1].

2.6. Intestinal glucose absorption

Two-hour fasted mice were euthanized. The duodenum was then harvested, washed, everted, and filled with in a Krebs–Ringer solution without glucose. Everted duodenal sacs were incubated in Krebs–Ringer with 10 g/L of glucose alone or with drugs, for 2 min at 37 °C [5]. The media of each sac was then collected and immediately frozen for subsequent galanin and glucose quantification studies. Glucose was measured using Glucose GOD FS 10'kit (DiaSys, France).

