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Leptin modulates enteric neurotransmission in the rat proximal colon: An in vitro study

Voinot Florian, Fischer Caroline, Crenner Francis, Schmidt Camille, Angel Fabielle *

Université de Strasbourg, Institut Pluridisciplinaire Hubert Curien, 23 rue Becquerel, 67087 Strasbourg, France
CNRS, UMR7178, 67037 Strasbourg, France

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ABSTRACT

Leptin has been shown to modulate gastrointestinal functions including nutrient absorption, growth, and inflammation and to display complex effects on gut motility. Leptin receptors have also been identified within the enteric nervous system (ENS), which plays a crucial role in digestive functions. Although leptin has recently been shown to activate neurons in the ENS, the precise mechanisms involved are so far unknown. Therefore, the aim of the present study was to determine the effects of leptin on rat proximal colon smooth muscle and enteric neuron activities. The effects of exogenous leptin on tone and on responses to transmural nerve stimulation (TNS) of isolated circular smooth muscle of proximal colon in rats were investigated using an organ bath technique. The effects of a physiological concentration (0.1 μM) of leptin were also studied on tone and TNS-induced relaxation in the presence of atropine, hexamethonium, L-N^G-nitroarginine methyl ester (L-NAME) and capsazepine. Leptin caused a slight but significant decrease in tone, TNS-induced relaxation and contraction in a concentration-dependent manner in colonic preparations. Cholinergic antagonists abolished the effects of 0.1 μM leptin on TNS-induced relaxation. This concentration of leptin had no further effect on relaxation in the presence of L-NAME. In the presence of capsazepine, leptin had no further effect either on tone or relaxation compared to the drug alone. In conclusion, leptin modulates the activity of enteric inhibitory and excitatory neurons in proximal colon. These effects may be mediated through nitrergic neurons. Intrinsic primary afferent neurons may be involved.

1. Introduction

White adipose tissue, apart from its role in energy storage, is a target for various hormones and is an active endocrine organ producing adipokines. These substances are polypeptides acting on various tissues and organs (including muscles, liver and hypothalamus) and they are involved in the physiopathology of metabolic regulations such as energy homeostasis, glucose and lipid metabolism, blood pressure regulation, immune functions and inflammation [1–4].

Leptin was the first adipokine that has been shown to be involved in the regulation of metabolism [5,6], food intake and energy balance [7]. Indeed, it acts as a sensor of fat mass in part of a negative feedback loop that maintains a set point for body fat stores [8]. Leptin is a 16 kDa non-glycosylated peptide hormone encoded by the gene *obese* (*ob*) [5]. It is predominantly produced by mature adipocytes but is also produced in other organs such as the stomach, placenta, skeletal muscle and mammary epithelium [9–12]. The plasmatic levels of leptin are highly correlated with the mass of visceral adipose tissue [13,14]. Leptin exerts its biological action in the brain through the activation of its receptor in the hypothalamus [15,16]. Because of the ubiquitous tissue distribution of its receptor, leptin also plays a role in several peripheral tissues, including skeletal muscle, liver, adipose tissue and intestine [17,18].

Within the gastrointestinal (GI) tract, the epithelium of the stomach is the major source of leptin [11,19]. Both endocrine and exocrine cells produce leptin. Leptin secreted in the gastric lumen remains stable despite the acidic environment and reaches the intestine in an active form. Leptin receptor isoforms have been found in the digestive tract from duodenum to colon, mainly in the basolateral membrane but also on the luminal border of enterocytes and colonocytes [20–22]. The presence of leptin receptors along the GI tract suggests that leptin may be involved in diverse functions [23]. Thus, leptin has been shown to modulate GI nutrient absorption, growth and inflammation [24]. Moreover, leptin is known to display complex effects on motility of the GI tract [24]. Leptin-deficient mice, which exhibit metabolic disturbances similar to obesity, showed an increase in gastric emptying and proximal intestinal transit but a decrease in overall intestinal transit [25,26]. This effect of leptin on GI motility may be explained by the presence of leptin

Abbreviations: ACh, acetylcholine; C, contraction; CCK, cholecystokinin; CMPC, circular muscle from proximal colon; ENS, enteric nervous system; GI, gastrointestinal; IPANs, intrinsic primary afferent neurons; L-NAME, L-NG-nitroarginine methyl ester; NO, nitric oxide; NOS, nitric oxide synthase; R, relaxation; TNS, transmural nerve stimulation.

* Corresponding author at: Département d'Ecophysiologie et Ethologie, Institut Pluridisciplinaire Hubert Curien, 23 rue Becquerel, 67087 Strasbourg Cedex 2, France. Tel.: +33 3 88106929; fax: +33 3 88 10 69 06.
E-mail address: fabielle.angel@iphc.cnrs.fr (A. Fabielle).

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receptors on vagal afferent neurons which transmit information to the central nervous system and then provide in return parasympathetic control for most visceral organs [27,28]. Moreover, leptin has been shown to cause excitatory and inhibitory effects on intestinal vagal mechanoreceptors and to stimulate intestinal motility in the presence of cholecystokinin (CCK) in cats [29,30].

The enteric nervous system (ENS) plays a crucial role in regulating digestive functions including motility and nutrient absorption. Because leptin receptors have been found in the antral and intestinal ENS of rodents, the effects of leptin on gut motility and nutrient absorption might be due to modulation of ENS activities [31,32]. Thus, leptin was shown to hyperpolarize glucoresponsive enteric neurons in guinea-pig [31] and recently to activate colonic submucous and myenteric plexus [33]. However, the precise mechanisms involved are so far unknown.

Therefore, the aim of the present study was to decipher the effects of leptin on rat colonic enteric neuron activities, as well as to pharmacologically characterize leptin mechanisms of action. Emphasis will lie on nitrergic, cholinergic and capsazepine-sensitive neurons which have been shown to be principally involved in transmission within the ENS [34,28,35].

2. Materials and methods

2.1. Animals and technique

Male Wistar rats (Charles River Laboratories, Lyon, France) were housed two per cage in a controlled environment at an ambient temperature of 24 ± 2 °C and under a 12 h light/dark cycle (lights on at 07.00 am). They had ad libitum access to food (standard low-fat diet A03, SAFE, Augy, France) and tap water. The day of experiment, rats (300–500 g) were anesthetized with isoflurane 2% (Centravet, Nancy, France) and were killed by decapitation. The proximal colon was immediately removed and immersed in a Petri dish containing a chilled physiological Krebs solution (in mM: 119.8 NaCl, 16.2 $C_6H_{12}O_6$, 15.5 $NaHCO_3$, 5.8 KCl, 2.5 $CaCl_2 \cdot H_2O$, 2.0 $NaH_2PO_4 \cdot H_2O$, 1.2 $MgCl_2 \cdot H_2O$, pH 7.4) oxygenated with 95% O_2/5% CO_2. Then, 8 strips of circular muscle (3 mm) were dissected from proximal colon (CMPC), rinsed of intraluminal content and mounted in organ bath chambers containing 10 mL of warmed (37 °C) and gassed (95% O_2/5% CO_2) Krebs solution. CMPC mechanical activities were measured by means of an isometric force transducer (Fort25, WPI, Aston, UK) and amplifier (Lab-Trax-4/24T, WPI, Aston, UK) and were visualized using a computer (HP Compaq 8000, Hewlett-Packard, USA). Transmural nerve stimulation (TNS) was applied via two steel electrodes, placed 10 mm apart from the tissue. Square wave pulses of 15 V intensity, 10 Hz frequency and 200 μs pulse duration, during a 10 s period of time, were used (stimulator designed by the Metrology and Instrumentation in Biology and Environment (MIBE) Department, IPHC, CNRS, Strasbourg, France).

Animal care was provided according to protocols and guidelines approved by the local Animal Care and Ethic Committees (CREMEAS #AL/06/25/12/09).

2.2. Experimental design

At the beginning of the experiment, each muscle strip was stretched incrementally to its optimal length, until the spontaneous contractile activity (basal tone and spontaneous rhythmic contractions) and the contractile responses to TNS were stable. Strips without spontaneous contractile activity or response to TNS were excluded from the study. Strips were finally allowed to equilibrate for 60 min after which the effects of various pharmacological agents on basal tone and responses to TNS were investigated. Bath solution was replaced every 15 min.

In a first series of experiments, three increasing concentrations of leptin (0.01, 0.1 and 1 μM) were added to the bath with washouts between each concentration. The pharmacological effects of each concentration were studied 10 min after its application. In a second series of experiments, the response to 0.1 μM of leptin (0.395 $\mu g \cdot mL^{-1}$) was studied in the presence of several pharmacological agents: acetylcholine (ACh, 10 μM), atropine (1 μM), hexamethonium (0.1 mM), L-N^G-nitroarginine methyl ester (L-NAME, 0.1 mM) and capsazepine (10 μM). ACh was added to the bath 10 min after the addition of leptin whereas the other drugs were administrated 10 min prior to leptin. After each experiment, tissues were washed out by replacing the bath solution several times with fresh Krebs solution and muscle strips were allowed to equilibrate for 30 min. Recovery of smooth muscle strips was regularly verified by stimulating enteric nerves.

2.3. Drugs

Leptin (human leptin) was purchased from Polypeptide Laboratories (Strasbourg, France), and atropine and hexamethonium from Sigma Aldrich (Saint-Quentin Fallavier, France). The other drugs were purchased from Tocris Bioscience (Bristol, UK). Capsazepine was dissolved in DMSO whereas the other substances were dissolved in distilled water. Solvent vehicles were added to the bath (in a volume of 100 μL in 10 mL) for control experiments. They showed no effect on spontaneous contractile activity or response to TNS developed by rat CMPC.

2.4. Data analysis

Analysis was performed using Datatrax (Datatrax 2.0, WPI, Aston, UK). Tone was evaluated for 2 min before TNS. The parameters of the TNS-induced response were measured as the area under the curve (in $g \cdot s^{-1}$, Fig. 1). The effects of the pharmacological agents are given as the percentage change from control basal tone or from area under the curve for TNS responses (defined as 100%; Fig. 1).

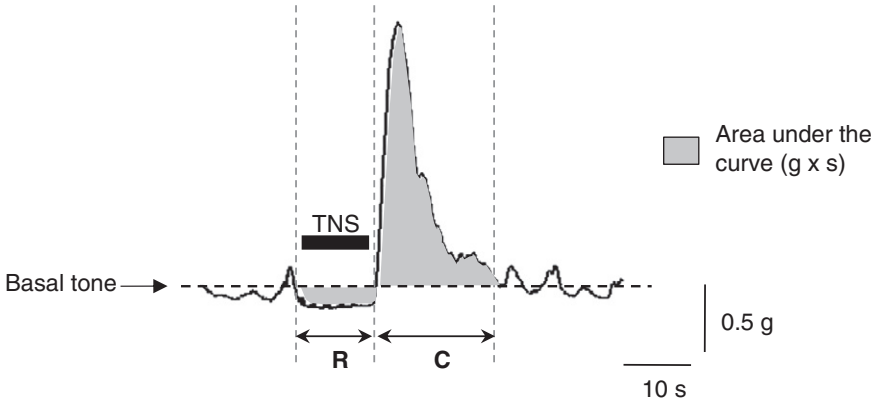

Fig. 1. Typical traces illustrating the mechanical response to transmural nerve stimulation (TNS) developed by CMPC in normal Krebs solution in rats. Mechanical response to TNS is composed of a relaxation (R) and an off-contraction (C) at the end of stimulation.