Neuroscience Letters 637 (2017) 85–90

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Infusion of fluoxetine, a serotonin reuptake inhibitor, in the shell region of the nucleus accumbens increases blood glucose concentrations in rats

C. Diepenbroek[a,1], M. Rijnsburger[a,1], L. Eggels[a], K.M. van Megen[a], M.T. Ackermans[c], E. Fliers[a], A. Kalsbeek[a,b], M.J. Serlie[a], S.E. la Fleur[a,*]

[a] Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
[b] Department of Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience, an institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, the Netherlands
[c] Department of Clinical Chemistry, Laboratory of Endocrinology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

HIGHLIGHTS

- Fluoxetine infused in the nucleus accumbens shell (sNAc), increases blood glucose.
- Fluoxetine's effects were partly explained by increased glucose production.
- Effect of fluoxetine in sNAc is independent of glucoregulatory hormones changes.
- We show first support for a role of the cortico-limbic system in glucose metabolism.

ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form
16 November 2016
Accepted 22 November 2016
Available online 22 November 2016

Keywords:
Nucleus accumbens shell
Glucose
Glucagon
Insulin
Endogenous glucose production

ABSTRACT

The brain is well known to regulate blood glucose, and the hypothalamus and hindbrain, in particular, have been studied extensively to understand the underlying mechanisms. Nuclei in these regions respond to alterations in blood glucose concentrations and can alter glucose liver output or glucose tissue uptake to maintain blood glucose concentrations within strict boundaries. Interestingly, several cortico-limbic regions also respond to alterations in glucose concentrations and have been shown to project to hypothalamic nuclei and glucoregulatory organs. For instance, electrical stimulation of the shell of the nucleus accumbens (sNAc) results in increased circulating concentrations of glucose and glucagon and activation of the lateral hypothalamus (LH). Whether this is caused by the simultaneous increase in serotonin release in the sNAc remains to be determined. To study the effect of sNAc serotonin on systemic glucose metabolism, we implanted bilateral microdialysis probes in the sNAc of male Wistar rats and infused fluoxetine, a serotonin reuptake inhibitor, or vehicle after which blood glucose, endogenous glucose production (EGP) and glucoregulatory hormones were measured. Fluoxetine in the sNAc for 1 h significantly increased blood glucose concentrations without an effect on glucoregulatory hormones. This increase was accompanied by a higher EGP in the fluoxetine infused rats compared to the controls. These data provide further evidence for a role of sNAc-serotonin in the regulation of glucose metabolism.

Abbreviations: ACh, Acetylcholine; cNAc, core region of the nucleus accumbens; DBS, deep brain stimulation; EGP, endogenous glucose production; NAc, nucleus accumbens; LH, lateral hypothalamic area; SSRI, shell region of the nucleus accumbens; sNAc, shell region of the nucleus accumbens; T2DM, type 2 diabetes mellitus; WAT, white adipose tissue.

* Corresponding author at: Department of Endocrinology & Metabolism, Academic Medical Center, University of Amsterdam, Meibergdreef 9, F2–131–2, 1105 AZ Amsterdam, the Netherlands.
E-mail address: s.e.lafleur@amc.uva.nl (S.E. la Fleur).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.neulet.2016.11.045
0304-3940/

1. Introduction

The ongoing obesity epidemic, leading to an increased risk for disturbances in glucose metabolism, led to regain of scientific interest in the physiology of glucose homeostasis. In addition to glucoregulatory hormones, such as insulin and glucagon, which decrease and increase blood glucose concentrations respectively, the brain has been shown to play an important role in regulating glucose metabolism [1]. The brain orchestrates glucose metabolism through innervation of glucoregulatory organs like liver, skeletal muscle and adipose tissue, that are involved in endogenous glucose production (EGP) and glucose uptake [2–4]. Moreover, central innervation of beta-cells in the pancreas have been described providing a route via which the brain influences insulin and glucagon secretion [5,6]. The classical view of glucose control by the hypothalamus and hindbrain has recently been expanded to include other brain areas [7]. For example, the nucleus accumbens (NAc), which is part of the reward circuitry controlling feeding behaviour, contains glucose sensing cells [8], and provides input to the lateral hypothalamus (LH) [9], an area implicated in glucose regulation [10,11]. The NAc consists of a core (cNAc) and a shell (sNAc) region, which are functionally different, for instance, with regard to feeding behaviour, and [12,13] input and output areas [14]. We recently showed that electrically stimulating the sNAc using deep brain stimulation (DBS) increases plasma concentrations of glucose and glucagon and activated neurons in the LH [15], pointing to a role for the sNAc in control of glucose homeostasis. It remains, however, to be determined which neurotransmitter system in the sNAc underlies these changes.

The NAc receives dense input from different areas in the brain involved in energy metabolism, and is, for example, innervated by the serotonergic neurons from the raphe nuclei and periaqueductal grey [16] forming synaptic contacts with cells in the sNAc particularly [17]. Central serotonin has been shown to control glucose metabolism [18], and others have shown that DBS in the rat sNAc (and not in the cNAc) increases local serotonin and dopamine concentrations [19].

To determine the effect of elevated extracellular serotonin concentrations in the sNAc on glucose metabolism, we bilaterally implanted rats with micro dialysis probes aimed at the sNAc and subjected rats to 1 h reverse micro dialysis with the selective serotonin reuptake inhibitor (SSRI) fluoxetine, which has been shown to increase extracellular serotonin concentrations [20,21]. We assessed EGP and concentrations of blood glucose and the glucoregulatory hormones glucagon, insulin and corticosterone. This study shows for the first time that infusing a serotonin reuptake inhibitor in the sNAc increases blood glucose concentrations, partly explained by changes in EGP but not glucoregulatory hormones. These findings are in line with earlier findings that the sNAc plays a role in glucose metabolism [15].

2. Material and methods

2.1. Animals

Male Wistar rats (250–280 g Harlan, Horst, the Netherlands) were individually housed in Plexiglas cages in a temperature ($20 \pm 2\,^{\circ}C$), humidity ($60 \pm 2\%$) and light controlled room with a 12/12 h light-dark schedule (lights on at 7:00 AM). All animals had *ad libitum* access to laboratory chow (Teklad Global 18% Protein Rodent Diet, Harlan, Horst, the Netherlands) and tap water prior to testing.

After arrival at the animal facilities, rats received one week for acclimatization during which they were adapted to handling. The experiment was approved by the Committee for Animal Experimentation of the Academic Medical Centre of the University of Amsterdam, the Netherlands.

2.2. Surgery

Rats were anaesthetized with an ip. injection of a mixture of 80 mg/kg Ketamine (Eurovet Animal Health, Bladel, the Netherlands), 8 mg/kg Rompun® (xylazine, Bayer Health Care, Mijdrecht, the Netherlands) and 0.1 mg/kg Atropine (Pharmachemie B.V., Haarlem, the Netherlands), after which a silicone catheter was implanted in the jugular vein, according to the method of Steffens (1969), for intravenous (iv.) infusions. Another silicone catheter was implanted in the left carotid artery for blood sampling. After catheter implantations, rats were fixed in a stereotact (Kopf®, David Kopf instruments, Tujunga, California) and micro dialysis probes (molecular weight cut-off 6 Kda, U-shaped tip 1.5 mm long, 0.7 mm wide, and 0.2 mm thick; constructed as reported in [22]) were bilaterally implanted in the sNAc (AP + 1.44 mm, ML ±3 mm, DV −7.3 mm, angle 17°). Catheters and micro dialysis probes were fixed on the skull with dental cement. Rats received Carprofen (5 mg/kg BW, subcutaneous) during surgery and the first post-surgery day, and a recovery period of 14 days in which food and water intake and body weight were measured 5 times a week. Jugular vein catheters were flushed twice a week.

2.3. Fluoxetine concentration

Fluoxetine (fluoxetine hydrochloride, Sigma Aldrich, Germany) was dissolved in Ringer (73.5 Na^{2+}, 2 K, 1.13 Ca^{2+}, 77.8 Cl in mmol/500 mL; Baxter, Utrecht, the Netherlands) to a concentration of 250 μM. We chose the maximum dose of 250 μM based on previous studies using *in vivo* reverse micro dialysis of fluoxetine in the NAc in rats [21,23]. Also, we performed a pilot experiment including 3 groups infusing either vehicle (Ringer), fluoxetine 83 μM or fluoxetine 250 μM. The probes were correctly placed in 4 animals in the 83 μM fluoxetine group and 4 in the vehicle group. Plasma glucose and EGP were not different between the 83 μM dose fluoxetine group and the vehicle group. We therefore performed the experiment, described in this report, with the 250 μM fluoxetine concentration.

2.4. Glucose kinetics and reverse micro dialysis

On the evening prior to the experiment, rats were connected to a multi-channel fluid infusion swivel (Instech Laboratories, PA, USA) to adapt. Food was restricted to 20 g of chow to avoid differences in the nutritional state and basal blood glucose concentrations of the rats.

On experimental days, remaining food was removed at the beginning of the light period (8:00 AM), which was 2 hours before the experiment started. Subsequently, animals were connected to the blood-sampling catheter and micro dialysis lines which were, via the multi-channel fluid infusion swivel, connected to an infusion pump (Harvard Apparatus, Holliston, Massachusetts, USA). The sampling catheter and cables were kept out of reach by means of a counterbalanced beam. This allowed the animals to move freely during the experiment and allowed all manipulations to be performed outside the cages without handling the animals.

To study glucose kinetics, [6.6-$^{2}H_2$] glucose was used as a tracer (>99% enriched; Cambridge Isotope Laboratories, Cambridge, USA). Five minutes prior to infusion of the stable isotope tracer, a blood sample was drawn to assess background isotopic enrichment. At 10:00 AM a primed (3000 μL/h in 5 min (=250 μL)) followed by a continuous [6.6-$^{2}H_2$] glucose (>99% enriched; Cambridge Isotope Laboratories, Cambridge, USA) (500 μL/h) infusion was started using the infusion pump. After 90 min of equilibration time, three