Life Sciences xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Life Sciences

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

# Inhibitory effect of visfatin and leptin on human and rat myometrial contractility☆

Sadaf Mumtaz *, Seham AlSaif, Susan Wray, Karen Noble

*Department of Cellular & Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Crown Street, Liverpool, L69 3BX, UK*

ARTICLE INFO

*Article history:*
Received 20 October 2014
Accepted 23 January 2015
Available online xxxx

*Chemical compounds studied in this article:*
Leptin (PubChem CID: 19625677)
Visfatin (PubChem CID: 15604363)

*Keywords:*
Maternal obesity
Contractions
Adipokine
Smooth muscle
Caesarean

ABSTRACT

*Aims:* The purpose of this study was to investigate the effect of visfatin on *in vitro* myometrial contractility in human and rat, and compare it to leptin.
*Main methods:* Myometrial strips from term pregnant women having a caesarean section or rats were dissected, superfused with physiological saline and the effects of visfatin (500pM–25nM) or leptin (1nM–1μM), on spontaneous and oxytocin-induced contractions were studied. After establishment of regular contractions, tissues were incubated for control and test response at 37 °C for 20 min, and then contractility was assayed.
*Key findings:* In human and rat myometrium, visfatin had similar dose dependent effects on contractility. In the human myometrium, compared with that of controls (100%), 10 nM produced a significant (paired t-test) decrease in the 20 min integral of spontaneous (64 ± 8%, n = 13) and oxytocin-induced contractions (55 ± 9%, n = 5), mean ± SEM. In rat tissue the decrease was also significant (spontaneous, 76 ± 7%, n = 7; oxytocin-induced 68 ± 6%, n = 3). Leptin at this concentration (10 nM) had no effect in rat or human, and even at a higher concentration (1 μM) produced only a small inhibitory effect (~ 80%) on contractions.
*Significance:* These data are the first to show that visfatin inhibits myometrial contractility and does so more potently than leptin. Our data suggest that increased output of visfatin and leptin in obese pregnant women may impair uterine contractility resulting in an unplanned Caesarean delivery.


## Introduction

In many parts of the world Caesarean section (CS) has reached epidemic proportions particularly in women having their first child [1]. In approximately half of unplanned CS, the most common clinical diagnosis is failure or slow progress in labour, also known as dystocia. Coinciding with the increased CS rate has been an increase in women who are overweight or obese [2]. This has led to investigators hypothesizing that obesity may compromise the ability of the labouring uterus to contract [3–6]. The dyslipidaemia associated with obesity, particularly elevated cholesterol levels may be a causal factor, as cholesterol inhibits uterine contractility and calcium signalling [7,8]. Data also shows increased rates of post-dated pregnancies and induction, and a decrease in the rate of spontaneous pre-term delivery in obese women [9–11].

In addition to dyslipidaemia, the metabolic and endocrine/paracrine products of adipose tissue exert diverse biological actions, including effects on smooth muscle contractility. Adipokine receptors are found in many sites throughout the body. Leptin, a hormone that regulates appetite and energy metabolism, is synthesized in adipose tissue and is under the control of obesity genes [12]. However it is also synthesized by the placenta [13,14], and its levels increase in maternal blood during pregnancy [15,16]. Its receptor is expressed in human umbilical cord, foetal membrane and uterine tissue. Leptin has been identified as a regulator of ovarian function, embryo development, and implantation [17].Of particular note, leptin has been shown to reduce contractions of human myometrium [18,10]. The question therefore arises whether other adipokines may also inhibit uterine contractility, and if others may be more potent and capable of targeting, to affect labour outcome.

Visfatin, PBEF (pre-B-cell colony-enhancing factor) and NAMPT (nicotinamide phosphoribosyltransferase) are the same protein separately identified: (i) *visfatin* is an adipokine, highly expressed in visceral fat and exerting insulin mimicking effects through the activation of an insulin receptor [19–22], (ii) *PBEF* is the secreted growth factor that enhances B-cell precursor maturation, and (iii) *NAMPT* catalyses the first rate-limiting step in converting nicotinamide to NAD(+), a key enzyme in nicotinamide adenine dinucleotide (NAD) generation, crucial for cellular metabolism and energy production [23]. Visfatin/NAMPT can be found intra- and extracellularly. We will call this protein visfatin henceforth when discussing it as an adipokine.

Visfatin is constitutively expressed in the myometrium [24], as well as placenta [25], and human foetal membranes [26]. Its gene is

☆ Support for this research work was granted by the Commonwealth Scholarship Commission, UK, to Sadaf Mumtaz.
* Corresponding author at: College of Medicine, University of Dammam, Saudi Arabia. Tel.: +966 590010877.
*E-mail address:* amumtaz@uod.edu.sa, dr_sadaf_mumtaz@yahoo.com (S. Mumtaz).

http://dx.doi.org/10.1016/j.lfs.2015.01.020
0024-3205/

upregulated in the foetal membranes in response to mechanical stretch and term or preterm labour [27,26]. Parturition also causes increased gene expression from the myometrium [24,28], as well as the membranes, resulting in an increase in its protein in maternal serum [24, 29,30,31]. There have however been no investigations of visfatin's effect on myometrium.

Due to the increase in the expression, serum levels and metabolic functions associated with leptin and visfatin during pregnancy, we hypothesize that leptin and visfatin may modulate myometrial function and activity. The aim of this study was therefore to investigate and compare the effects of visfatin and leptin on both spontaneous contractions and those elicited by agonist oxytocin, on human and rat myometrial tissue *in vitro*.

## Materials and methods

### *Tissue collection*

Biopsies of human myometrial tissues were obtained at elective caesarean section in term pregnancy, after informed consent, at Liverpool Women's Hospital, UK. The ethical standards for human experimentation established by the Declaration of Helsinki [32] were followed. The biopsies were excised from the lower uterine segment incision for human and rat. Previous work has shown that contractility and responses to agonists from this region are also representative of the upper segment [33]. The rats were mated at age 10–12 weeks and had a body weight of 180–220 g. Rat myometrial tissue was obtained from humanely killed late pregnant rats (days 20–21 of gestation) according to the UK Home Office legislative requirements. All tissue biopsies were placed in physiological saline at pH 7.4 containing the following (mmol/L): NaCl 120.4, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.0, glucose 8, and HEPES 11 (Sigma Aldrich, UK). Tissues were stored at 4 °C and used within 24 hrs of collection.

Biopsies of human myometrial tissue were obtained from a total of 22 non-labouring women, between 38 and 40 weeks of gestation and BMI between 23–35. The reasons for caesarean section were previous C-section, rhesus incompatibility and previous traumatic vaginal delivery. The mean maternal age at delivery was 32.8 years (range 23–43 years). The average parity value of the women at the time of delivery was 1 (range 0-2). Caesarean sections were carried out under regional anaesthesia.

### *Contractility measurements*

Longitudinal myometrial strips (~1 × 4 mm) were dissected and attached to aluminium clips, attached to a tension transducer under 2 mN of tension in an organ bath (5 ml) superfused with physiological saline at 2 ml/min. [34]. Experiments were performed at 37 °C. Myometrial strips were allowed to equilibrate for at least 1 hour to obtain reproducible spontaneous phasic or oxytocin (0.5 nM) induced contractions. Tissue was incubated for control and test response for 20 mins at 37 °C. Force recordings were made before and after incubation applying the same amount of tension *i.e.* 2 mN. Matched vehicle controls were performed with Tris-HCl. The effect of visfatin or leptin and the respective controls were analysed by calculation of the amplitude and integrals of force (AUC) for each 20 min. interval before and after incubation using OriginPro 9.0 software [35]. The inhibitory effects of visfatin or leptin were corrected for any reduction in the contractile activity observed in vehicle control after incubation, which was around 2%.

### *Drugs and solutions*

Stock solutions were made in 20 mM Tris–HCl, pH: 7.4, for visfatin (100 nM) and leptin (50 μM). A stock solution of oxytocin (10 μM) was made using sterile water. Serial dilutions were made in fresh saline solution on the day of experimentation. All chemicals were purchased from Sigma Aldrich, (UK), unless otherwise stated.

### *Statistical analysis*

Comparison of force records for visfatin or leptin were performed using mean ± SEM followed by a paired Student's t-test to determine significant differences between control and adipokine and unpaired t-test among data groups. A P value of $<0.05$ was considered to be statistically significant. The F test was used to compare the controls before and after incubation and it showed that there was no significant difference between them.

## Results

### *Effect of visfatin and leptin on spontaneous and agonist induced human myometrial contractions*

Initial experiments were performed with continuous perfusion to establish the effect of visfatin (10 and 100 nM), and an example is shown in Fig. 1A. Having established visfatin affected force, in order to reduce the large volume and associated high cost of superfusion, all subsequent data were obtained by incubating tissue with visfatin or leptin after a control period, and reassessing activity. Figs. 1B*(i)* & 3*(i)* show representative recordings of spontaneous and oxytocin-induced myometrial contractions, respectively, before and after incubation in a vehicle control solution. Controls (spontaneous or agonist-induced) before and after incubation were compared and no significant difference was found between them (n = 13). Visfatin exerted an inhibitory effect on both spontaneous and oxytocin induced contractions in human myometrium *in vitro*, in all strips. Figs. 1B*(ii)* &3*(ii)* demonstrate the

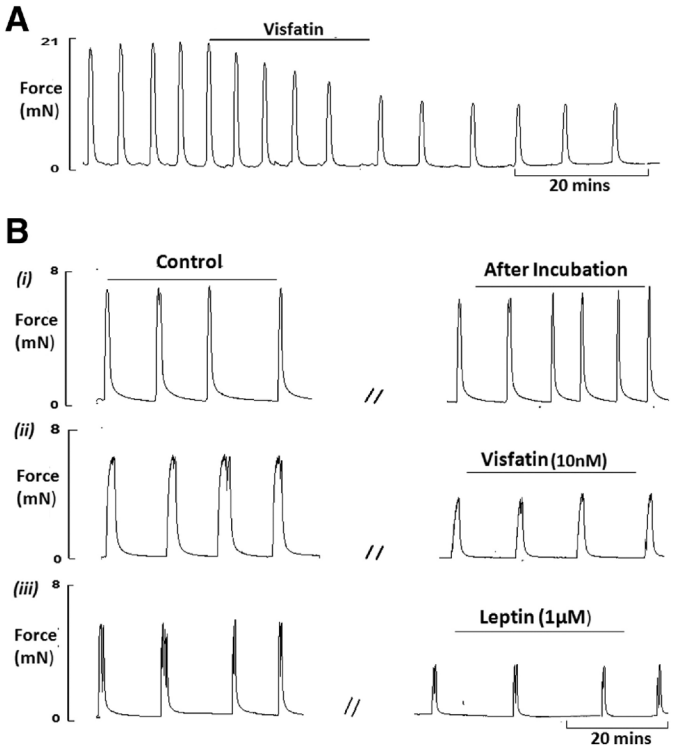


**Fig. 1.** (A) Effect of continuous perfusion of visfatin (100 nM) on spontaneous myometrial contractions in pregnant human non-labouring tissue. (B) Effect of visfatin and leptin on spontaneous myometrial contractility in pregnant human non-labouring tissue. Representative recordings of *(i)* spontaneous contractions in vehicle control strips, *(ii)* the effect of visfatin (10 nM), *(iii)* the effect of leptin (1 μM), before and after a 20 min incubation are shown.

Please cite this article as: S. Mumtaz, et al., Inhibitory effect of visfatin and leptin on human and rat myometrial contractility, Life Sci (2015), http://dx.doi.org/10.1016/j.lfs.2015.01.020