Life Sciences 116 (2014) 25–30

Contents lists available at ScienceDirect

Life Sciences

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

The effects of methyl palmitate, a putative regulator from perivascular fat, on the contractility of pregnant human myometrium

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ARTICLE INFO

Article history:
Received 1 July 2014
Accepted 27 August 2014
Available online 6 September 2014

Keywords:
Methyl palmitate
Pregnant human myometrium
Contractility
Perivascular fat
Voltage-gated potassium channels
4-Aminopyridine
Tetraethylammonium
Oxytocin
Dysfunctional labor
Obesity

ABSTRACT

Aims: Methyl palmitate is thought to cause relaxation in vascular smooth muscle by opening voltage-activated potassium channels. We have tested the hypothesis that methyl palmitate, a putative regulator from perivascular fat, is an inhibitor of the contractility of human pregnant myometrium and that its effects might partially explain the higher incidence of dysfunctional labor in obese women compared to those with normal body mass indices.
Main methods: Strips of myometrium obtained with informed consent from women undergoing elective cesarean section at term were mounted in organ baths. Strips stimulated with oxytocin (1 nM) or KCl (30 mM) were exposed to cumulatively increasing concentrations of methyl palmitate up to 10 μM. Similar strips were exposed to cumulative addition of the potassium channel blockers 4-aminopyridine and tetraethylammonium. The contractility of the strips was monitored and analyzed using conventional methods.
Key findings: Methyl palmitate failed to inhibit oxytocin- or KCl-induced contractions over the concentration range tested. In fact, it exerted a slight excitatory effect in the presence of KCl, though not in the presence of oxytocin. The contractility of naïve strips was unaltered by exposure to 1 μM methyl palmitate. Both 4-aminopyridine and tetraethylammonium produced concentration-dependent contractions of human pregnant myometrium providing pharmacological evidence for the presence of voltage-activated potassium channels in this preparation.
Significance: Our findings do not support the hypothesis that methyl palmitate is an inhibitor of human pregnant myometrial contractility. Alternate hypotheses must be pursued to explain the higher incidence of dysfunctional labor in obese women.

Introduction

A large body of clinical evidence supports the contention that maternal obesity is a major risk-factor for poor uterine contractility at term (Cnattingius et al., 1998; Sebire et al., 2001; Lynch et al., 2008). However, it is unclear whether there is an inherent contractile defect in the myometrium of obese women (Zhang et al., 2007; Higgins et al., 2009; Sweeney et al., 2013). One possible mechanism that would explain poor uterine contractility in obese women without changes in the myometrium itself is the dysregulation of mediators that contribute to the normal control of contractility. To date several compounds (leptin, apelin, ghrelin) that might be expected to increase during obesity have been shown to inhibit human uterine contractility in vitro (Moynihan et al., 2006; O'Brien et al., 2010; Hehir and Morrison, 2012).

Much recent interest has focused on perivascular adipose tissue (PVAT) (Lee et al., 2013). It is now clear that all blood vessels are surrounded by PVAT and that secretory compounds released from this tissue play a central role in cell signaling and regulation, both under normal physiological conditions, and in response to inflammation, tissue injury, obesity and aging (Campia et al., 2012; Miao and Li, 2012). In particular, it is now well established that contractility of the vasculature is regulated by mediators emanating from PVAT (Dubrovska et al., 2004; Gao et al., 2007; Malinowski et al., 2008). These mediators help to suppress vascular excitability, but in obesity they become dysregulated (Greenstein et al., 2009). While there is little or no knowledge available in relation to the potential regulation of the myometrium of any species by PVAT, some pathways that regulate the contraction of human myometrial arteries are impaired by obesity and it has been suggested that this impairment might be mediated by PVAT (Hayward et al., 2014).

Among the mediators released from PVAT are the polypeptide adiponectin (Lynch et al., 2013) and a broad array of lipids (Clària et al., 2013). While roles of adiponectin in reproductive processes have been suggested (Palin et al., 2012), little is known about its function in the myometrium. However it has recently been demonstrated that

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http://dx.doi.org/10.1016/j.lfs.2014.08.018
0024-3205/

both the adipo1 receptor and the adipo2 receptor are expressed at the gene and protein level in porcine myometrium (Smolinska et al., 2014) but their role is unclear, and to our knowledge there are no data pertaining to functional effects in pregnant human myometrium. Similarly, although effects of prostanoids and hydroxyeicosatetraenoic acids on the contractility of pregnant human myometrium are well-documented (Senior et al., 1993; Crankshaw and Dyal, 1994; Pearson et al., 2009) there is scant information on the effects of other lipid mediators.

Methyl palmitate (palmitic acid methyl ester, PAME) is another mediator released from PVAT (Lee et al., 2011). PAME produces a potent relaxation of rat aorta (EC_{50} = 0.8 pM) through a mechanism independent of nitric oxide synthesis that appears to involve the opening of 4-aminopyridine- and tetraethylammonium-sensitive K^+ channels (Lee et al., 2010, 2011). Because of its low molecular weight and hydrophobic nature, PAME released from the perivascular adipose tissue of uterine blood vessels may have the potential to defuse to the myometrium, and hence we hypothesized that it would inhibit human myometrial contractility by a similar mechanism.

Materials and methods

Biopsy specimens of human myometrial tissue were obtained at elective cesarean section as described previously (Crankshaw and Morrison, 2011). Recruitment of donors was by written informed consent and approval for tissue collection was obtained from the Research Ethics Committee at Galway University Hospital, Ireland.

Tissue bath experiments

Myometrial strips measuring approximately 2 × 2 × 10 mm were prepared from the biopsy specimens and mounted for isometric recording as previously described (Crankshaw and Morrison, 2011). After an equilibration time of 130 to 160 min strips were challenged with 30 mM potassium chloride (KCl). The KCl was left in contact with the tissue for a period of 10 min, after which baths were washed with physiological salt solution (PSS) and the tissues rested for a further 30 min. At the end of all experiments tissues were challenged with nifedipine (10 μM) to relax them to baseline and establish 100% inhibition.

Concentration–effect curves for the inhibition of oxytocin-induced (1 nM) contractions by PAME were performed as described previously (Crankshaw, 2001; Crankshaw et al., 2013). When oxytocin had been in contact with the tissues for 30 min, data were collected for 10 min to establish a control oxytocin response. Thereafter, PAME or vehicle was added cumulatively at 12-minute intervals in approximately log concentration increments beginning at 600 pM for PAME. A total of 5 additions were made. Data collected for 10 min beginning 2 min after each drug addition were considered to represent the response to the cumulative PAME concentration then present in the bath. Concentration–effect curves for the inhibition of KCl-induced (30 mM) contractions by PAME were performed in essentially the same manner except that the lowest concentration of PAME was 200 pM. Parallel strips were exposed to cumulative log concentration increases in nifedipine beginning at 100 pM to verify concentration-dependent relaxation in this model. The hypothesis that PAME might have paradoxical excitatory effects in human pregnant myometrium was tested by adding a single, high concentration of PAME (1 μM) to the baths at the end of the post-KCl rest period and recording activity for 80 min in the continued presence of PAME. At the end of this period oxytocin (1 nM) was added to ensure viability of the strips and contractility was recorded for a further 30 min in the presence of oxytocin. Concentration–effect experiments for 4-aminopyridine (4AP) and tetraethylammonium (TEA) were performed by cumulative addition as we have described for other excitatory agents (Crankshaw and Morrison, 2011). Five additions of 4AP were made in approximately log unit increments beginning at 600 pM. Four additions of TEA were made in approximately half log unit increments beginning at 30 μM.

Contractile activity measurements

Contractile activity was simultaneously recorded using PowerLab/8SP hardware and Chart V 4.0 software (AD Instruments, Hastings, UK). Mean contractile force (MCF) was determined for appropriate collection periods (Crankshaw and Morrison, 2011). Data were expressed either as a percentage of the activity during the control period or as a percentage of the initial KCl response as appropriate. The experiments were performed on a total of n = 67 myometrial strips dissected from n = 14 patient donor biopsies.

Data analysis

The MCF was calculated, corrections for time- and vehicle-matched controls were made, and curves were fitted as described previously

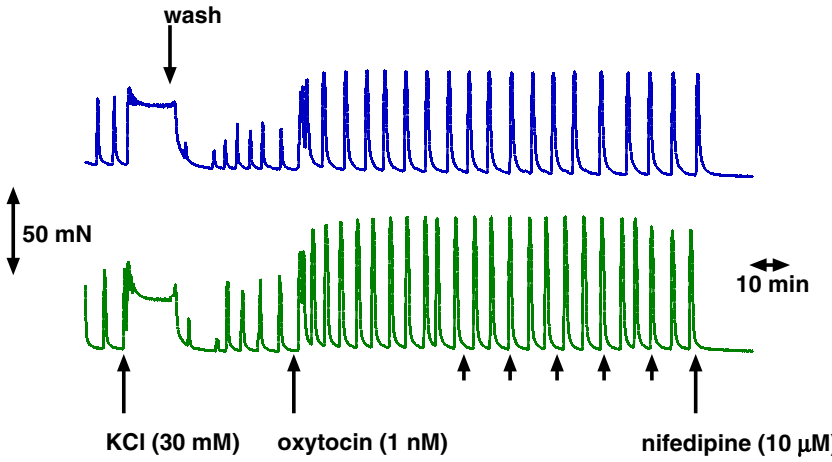

Fig. 1. Sample recordings showing the standard KCl response and then the effect of 1 nM oxytocin followed by the cumulative addition of PAME on the contractility of strips of human pregnant myometrium. Upper panel, vehicle control; lower panel, PAME. The unmarked arrows indicate points where cumulative additions were made in approximately log concentration increments beginning at 600 pM. The marked arrows indicate points where agents were added to both strips at the concentrations shown. Recordings are from parallel strips from the same donor.