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Original article

Participation of analogues of lysophosphatidic acid (LPA): oleoyl-sn-glycero-3-phosphate (L-α-LPA) and 1-oleoyl-2-*O*-methyl-*rac*--glycerophosphothionate (OMPT) in uterine smooth muscle contractility of the pregnant pigs

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Abstract

Recent studies show that a representative of phospholipids, namely lysophosphatidic acid (LPA) and its receptors (LPA_{1-3}) play a significant role in the reproductive processes, i. a, in the modulation of the uterine contractility. The participation of LPA_3 in the reproductive processes has been revealed in mice and has not been studied in gilts. Therefore, in the present study we investigated the role/action of LPA and its receptors LPA_1, LPA_2 and LPA_3 on the contraction activity in the porcine uterus. The study was conducted on an experimental model in which the pig uterus consisted of the one whole uterine horn and a part of the second horn, both connected with the uterine corpus. Uterine strips consisting of the endometrium with the myometrium (ENDO/MYO) and myometrium (MYO) alone were collected on days 12-14 of the estrous cycle (control group; n = 5) or pregnancy (experimental group; n = 5). Two analogues of LPA at increasing doses were used: oleoyl-sn-glycero-3-phosphate (L-α-LPA, a selective agonist of LPA_1 and LPA_2 receptors; 10^{-7} M; 10^{-6} M and 10^{-5} M) and 1-oleoyl-2-*O*-methyl-*rac*-glycerophosphothionate (OMPT, a selective agonist of LPA_3 receptor; 68 nM; 136 nM and 680 nM). L-α-LPA caused an increase in the contraction tension, amplitude and frequency of ENDO/MYO from the uterine horn with the developing embryos. This effect was not observed in MYO in both groups examined. In the ENDO/MYO strips of the uterine horn with developing embryos, OMPT significantly increased the contraction tension at the highest dose (680 nM) and amplitude at all doses examined, while frequency of contractions was decreased at doses of 136 nM and 680 nM. In the MYO strips of the uterine horn with embryos a significant increase in the contraction tension and amplitude after the highest dose of OMPT was observed. The results obtained imply the important role of receptors LPA_1, LPA_2 and LPA_3 in the contraction activity of the porcine uterus during early pregnancy.

Key words: gilts, uterine smooth muscle, LPA, OMPT, embryos

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Introduction

Spontaneous activity of the uterine myometrium, in terms of contraction tension, frequency and amplitude, is changing during the estrous cycle, mating or insemination, pregnancy and labour (Langendijk et al. 2002, 2005, Kurowicka et al. 2005). The contractile activity of the porcine myometrium is controlled by many factors including adrenergic, cholinergic and non-adrenergic non-cholinergic innervation (Łakomy 1987, Thilander et al. 1989, Taneike et al. 1991, Kaleczyc 1994, Majewski et al. 1995), as well as endocrine, paracrine and autocrine factors (Kitazawa et al. 1997, 2003, Cao et al. 2002, Sukjumlong et al. 2009, Franczak and Bogacki 2009). The most important substances regulating porcine uterine contractions include: noradrenaline (NA), serotonine (Kitazawa et al. 2001a), acetylcholine (Ach) (Kitazawa et al. 2003), oxytocin (Carnahan et al. 1999, Kitazawa et al. 2001b), prostanoids (prostaglandin $F_2\alpha$, E_1 and E_2) (Kucharski et al. 2007, Jana et al. 2010), tromboxane A_2, histamine (Kitazawa et al. 1997), neuropeptide Y (Markiewicz et al. 2003) and endothelin (Isaka et al. 2000). Recently it has been documented that besides the above-mentioned substances, a representative of phospholipids, such as lysophosphatidic acid (1-acyl-*sn*-glycerol-3-phosphate- LPA) and its receptor (LPA_3) may play a significant role in reproductive processes including modulation of the uterine contractility (Tigyi et al. 1992, Tokumura et al. 1999, Budnik and Mukhopadhyay 2002). LPA is a multifunctional lipid messenger and is the simplest of all glycerophospholipids (Moolenaar 1995). Lysophospholipase D is the enzyme responsible for LPA production (Xie and Meier 2004). This bioactive lipid mediator acts via G-protein-coupled receptors evoking multiple effects including Ca^{2+} mobilization, chemotaxis and cell proliferation (Tigyi et al. 1994, Liliom et al. 1996a, 1996b). LPA has been detected in different types of fluids including serum, plasma, saliva and follicular fluid (Tokumura et al. 1986, 1999, Tigyi and Miledi 1992). Recent identification and genetic manipulations of G-protein-coupled receptors specific to LPA (LPA_{1-3}) have provided mechanistic and functional insights into their diverse roles in biological processes (An et al. 1998, Bandoh et al. 1999, Contos et al. 2000a, 2002, Noguchi et al. 2003, Gardell et al. 2006). For example, targeted disruption of LPA_1 and LPA_2 has demonstrated their physiological and pathological functions in the neural development, neuropathic pain and diarrhea (Contos et al. 2000b, Inoue et al. 2004, Li et al. 2005). LPA_3 receptor is involved in the contractility of the myometrium in mice what determines evenly embryo spacing along the uterine horns (Hama et al. 2007). The increased expression of LPA_3 in the porcine endometrium during the periimplantation period indicates that it plays an important role during this stage of pregnancy (Ye et al. 2005, Kaminska et al. 2008). In the present study we investigated whether LPA and its analogues participate in the uterine contraction in gilts in early pregnancy. Considering the fact that in the porcine uterus the transcripts for LPA_1 and LPA_2 were also identified (Seo et al. 2008), we studied whether these isoforms may participatate in action of LPA on the porcine uterus contraction activity.

Materials and Methods

Prepubertal crossbred gilts (n=10) with an average body weight of 106 ± 4.8 kg and aproximately 7 months of age were used. The gilts were subjected to surgical procedure under general anesthesia. The animals were premedicated with azaperone (Stresnil, Janssen Animal Health; 2 mg/kg i.m.), ketamine (VetaKetam, Vet-Agro, Poland; 10-15 mg/kg i.m.) and anaesthetized with thiopental (Sandoz, GMBH, Austria 20-30 mg/kg i.v.) In five gilts (experimental group) the uterine horns were presented by a midventral opening of the caudal part of the abdomen as described previously (Wasielak et al. 2008). Briefly, one horn of each gilt was cut transversely and the ends were closed by a suture. This way an experimental model was created in which the pig uterus consisted of the one whole uterine horn and a part of the second horn, both connected with the uterine corpus. The remaining part of the second horn, connected with the contiguous ovary, was surgically detached from the uterine corpus. After 10 days of recovery from surgery, gilts were treated hormonally by an intramuscular injection of 750 I.U. PMSG (Folligon, Intervet, Poland) and 500 I.U. of hCG (Chorulon, Intervet) given 72 h later. Subsequently, gilts from the experimental group (n=5) were inseminated 24 h after the hCG treatment. The insemination was repeated twice at 12 h intervals. The remaining gilts (n=5; control group) were subjected to surgical procedure but not inseminated. At the Days 12-14 of pregnancy (experimental group) or estrous cycle (control group) gilts were slaughtered. To confirm pregnancy, the uterine horns were flushed with 10 ml PBS (pH=7.4) to determine the presence of embryos in uterine flushings (Wasielak et al. 2008). Fragments of the uterine horns, collected from the middle part of the horns, were transferred to ice and transported to the laboratory and immediately processed for examination of the contractile activity. The contractile activity was examined according to the method described previously (Jana et al. 2010). Briefly, two kinds of the uter-

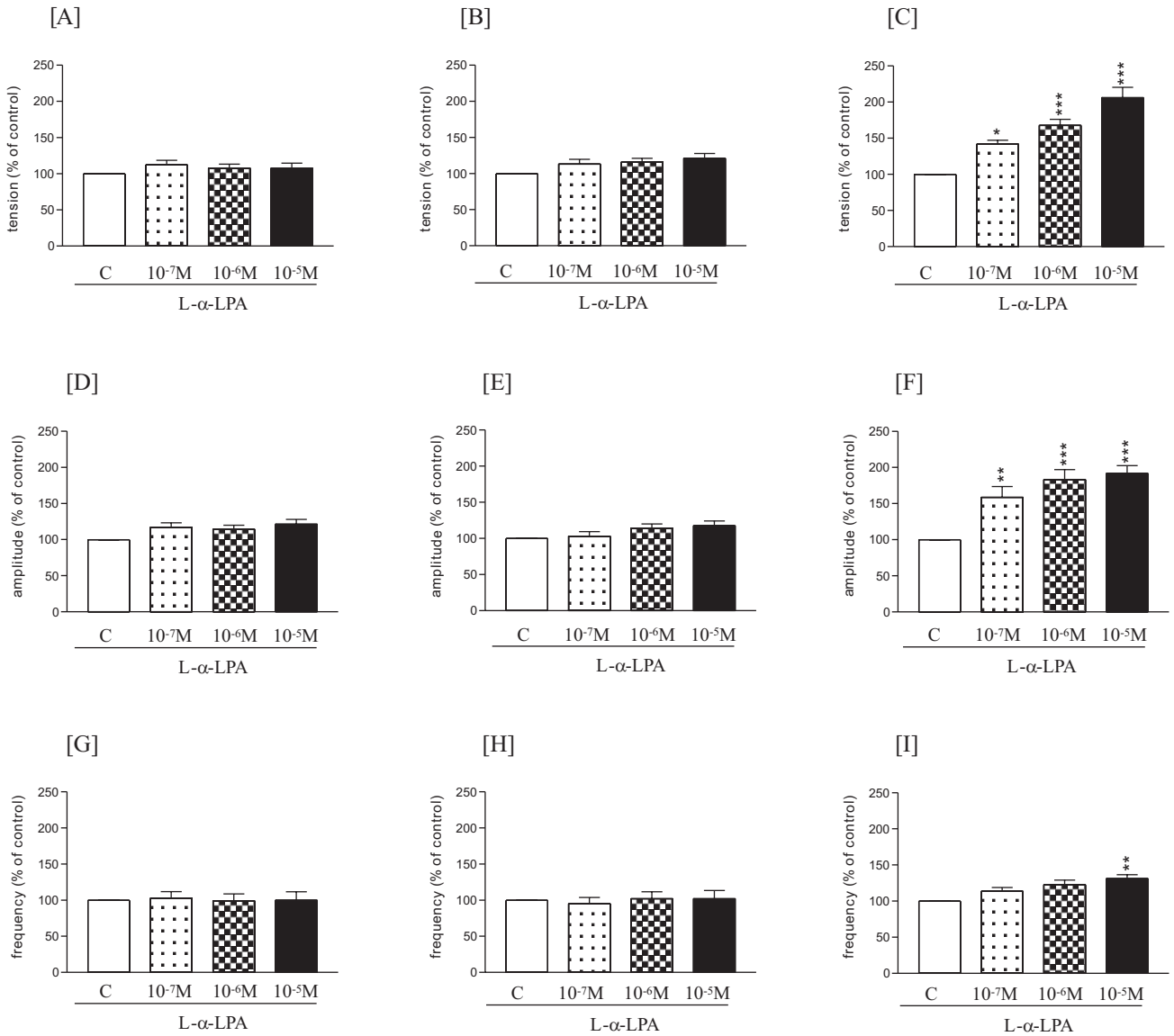

Fig. 1. Influence of oleoyl-sn-glycero-3-phosphate (L-α-LPA) on the tension ([A],[B],[C]) amplitude ([D],[E],[F]) and frequency ([G],[H],[I]) of the contraction of the porcine endometrium/myometrium strips of the control group ([A],[D],[G]), uterine horn without embryos ([B],[E],[H]) and with the embryos ([C],[F],[I]). Uterine strips were collected on days 12-14 of the estrous cycle (control group) or pregnancy (groups with embryos and without embryos). Values (mean ± SEM; n = 5 in each group) are expressed as a percentage of changes in the contractile activity before the treatment. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to the contractile activity before the treatment (C).

ine strips (3 × 5 mm) comprising the endometrium with the myometrium (ENDO/MYO) and myometrium (MYO) alone were resected, washed in saline and mounted between two stainless steel hooks in 5 ml of organ bath (Schuler Organ bath type 809; Hugo Sachs Electronic, Germany) under conditions of resting tension of 5 mN. The strips were kept in Krebs-Ringer solution of the following composition (mM/l): NaCl, 120.3; KCl, 5.9; $CaCl_2$, 2.5; MCl_2, 1.2; $NaHCO_3$, 15.5; glucose, 11.5; 37°C, pH 7.4. The solution was maintained at 37°C and continuously saturated with a mixture 95% O_2 and 5% CO_2. The recording was started after equilibration for at least 60 min. At the beginning of the experiment the strips were incubated with noradrenaline (Polfa, Poland) at dose of 10^{-6} M and acetylcholine (Sigma Aldrich, Germany) at a dose of 10^{-3} M to determine the viability of tissues and their usefulness to further study. Next, the effect of increasing concentrations of two analogues of LPA: oleoyl-sn-glycero-3-phosphate (L-α-LPA, a selective agonist of LPA_1 and LPA_2 receptors; 10^{-7} M; 10^{-6} M and 10^{-5} M; Sigma, USA) and 1-oleoyl-2-*O*-methyl-*rac*-glycerophosphothionate (OMPT, a selective agonist of LPA_3 receptor; 68 nM;

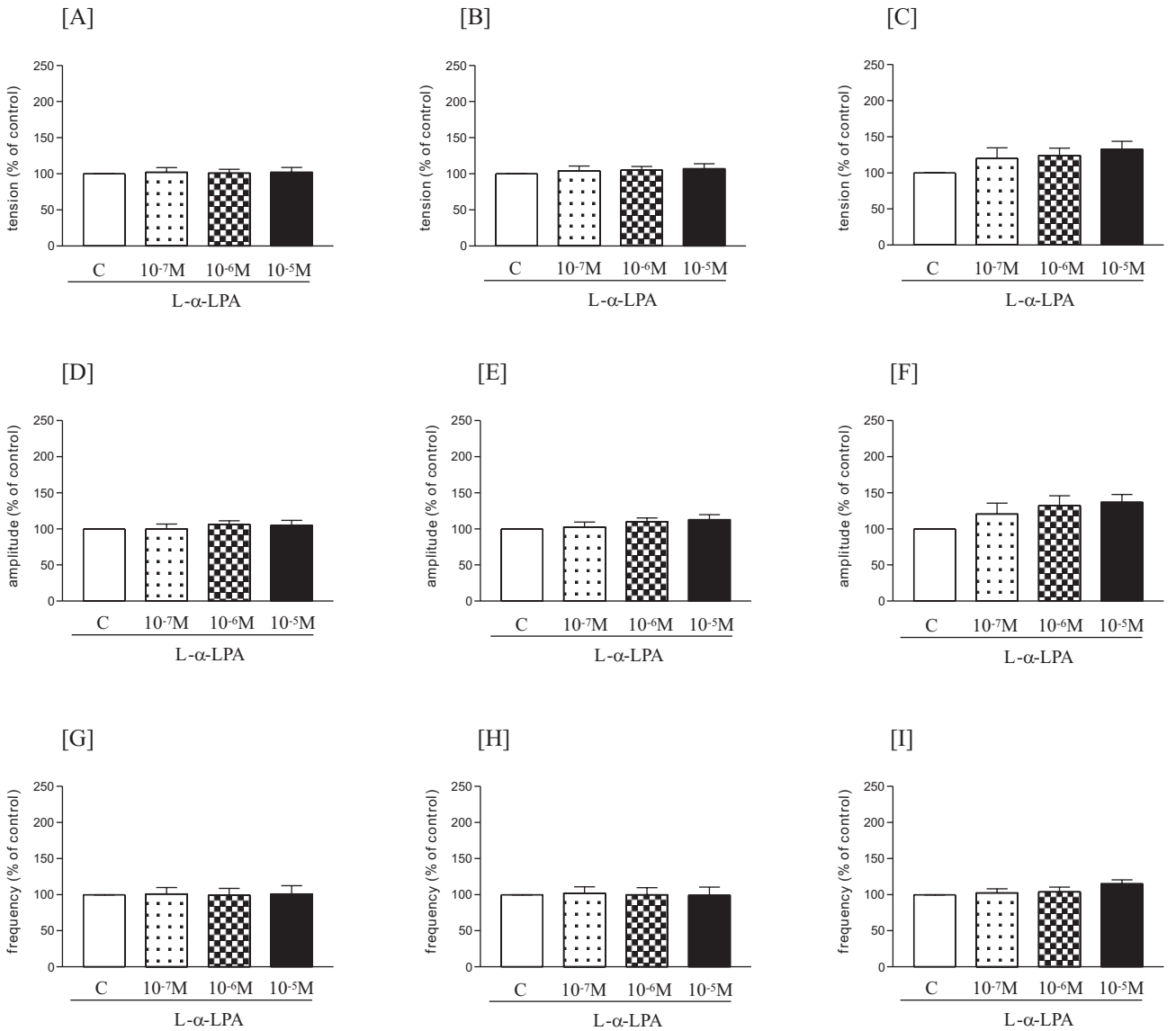

Fig. 2. Influence of oleoyl-sn-glycero-3-phosphate (L-α-LPA) on the tension ([A],[B],[C]) amplitude ([D],[E],[F]) and frequency ([G],[H],[I]) of the contraction of the porcine myometrium strips of the control group ([A],[D],[G]), uterine horn without embryos ([B],[E],[H]) and with the embryos ([C],[F],[I]). Uterine strips were collected on days 12-14 of the estrous cycle (control group) or pregnancy (groups with embryos and without embryos). Values (mean ± SEM; n = 5 in each group) are expressed as a percentage of changes in the contractile activity before treatment (C).

136 nM and 680 nM; Cayman Chemical, USA) on the contractile activity was studied. The doses of the substances tested were determined on the basis of the preliminary study. Contractile activity was measured for 10 min after administration of each concentration of the substance examined and thereafter, tissue chambers were washed three times with 15 ml of phosphate buffer at 10 min intervals. In the end, to determine the viability of tissues NA and ACh were administered at doses given above. In the statistical analysis only those results were considered, for which the difference in the response to the stimulation by NA and ACh at the beginning and end of the study was smaller than 20%.

The measurements of the smooth muscle contraction were conducted using a force transducer (HSE F-30 type 372), and bridge coupler type 570, and the graphic recording was made using recorder (Hugo Sachs Elektronik) with HSE-ACADW software. All procedures involving animals were conducted in accordance with the Local Research Ethics Committee (67/2009 of 29.09.2009) national guidelines for agricultural animal care.

Statistical analysis

The numerical values of the contraction activity (tension, amplitude and frequency) of tissues before

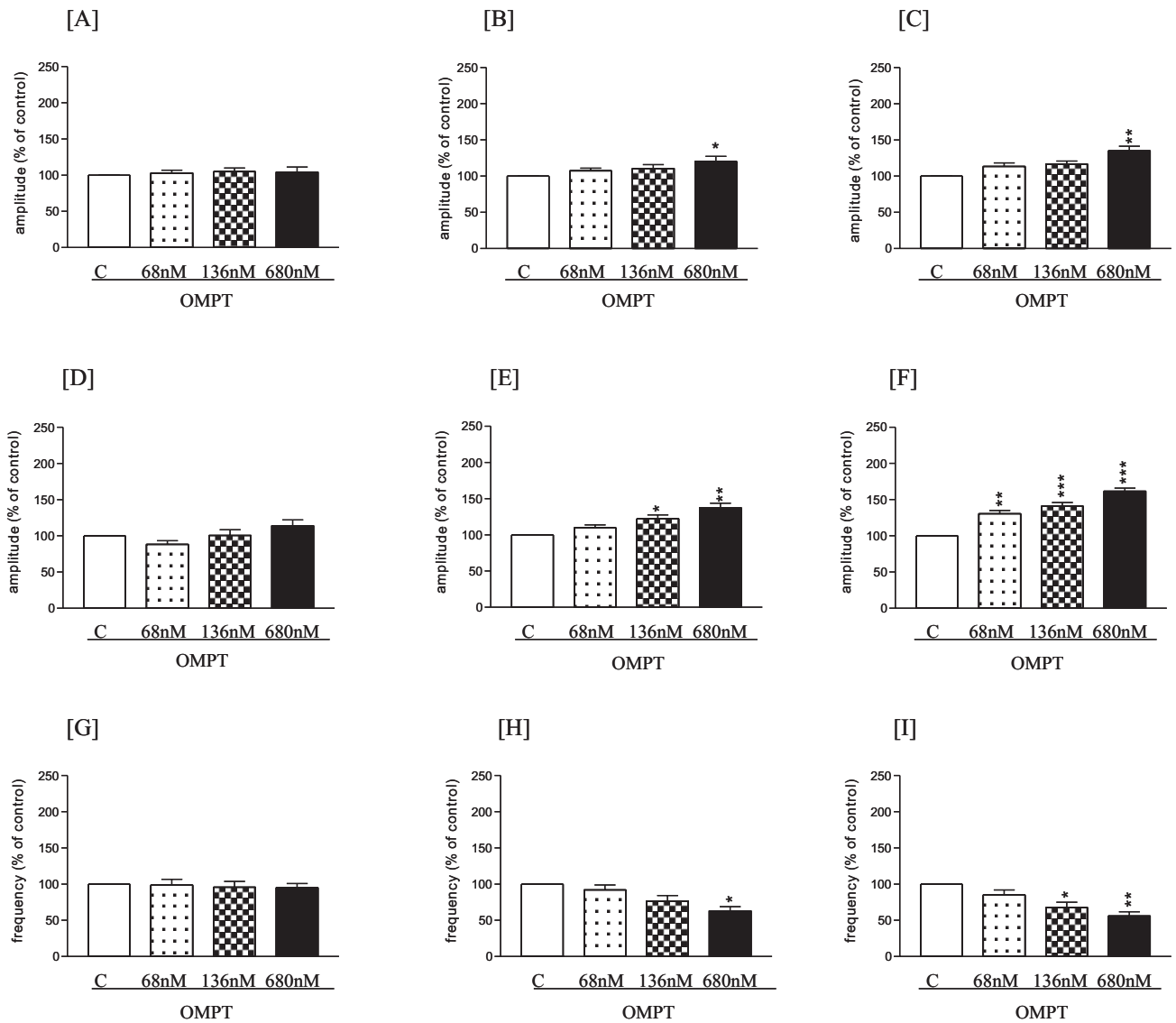

Fig. 3. Influence of 1-oleoyl-2-*O*-methyl-*rac*-glycerophosphothionate (OMPT) on the tension ([A],[B],[C]) amplitude ([D],[E],[F]) and frequency ([G],[H],[I]) of the contraction of the porcine endometrium/myometrium strips of the control group ([A],[D],[G]), uterine horn without embryos ([B],[E],[H]) and with the embryos ([C],[F],[I]). Uterine strips were collected on days 12-14 of the estrous cycle (control group) or pregnancy (groups with embryos and without embryos). Values (mean ± SEM; n = 5 in each group) are expressed as a percentage of changes in the contractile activity before the treatment. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to the contractile activity before the treatment (C).

the application of biologically active substances were calculated for 7 min and accepted as 100%. The results calculated for 7-min period after treatments were expressed as a percentage (mean ± SEM) of the contraction tension, amplitude and frequency before drug administration. The statistical significance of the differences obtained were assessed by one-way analysis of variance ANOVA (Graphpad PRISM 3.1; Graphpad Software, USA) followed by Bonferroni's Multiple Comparison Test. The differences at $P < 0.05$ were considered as statistically significant.

Results

Influence of L-α-LPA on the uterine contractile activity

In the ENDO/MYO uterine strips of the contol group and uterine horn without the embryos, L-α-LPA at all examined doses did not cause significant changes ($P > 0.05$) in the contraction tension, amplitude and frequency (Figs. 1, 5B). In the ENDO/MYO strips of the uterine horn with the

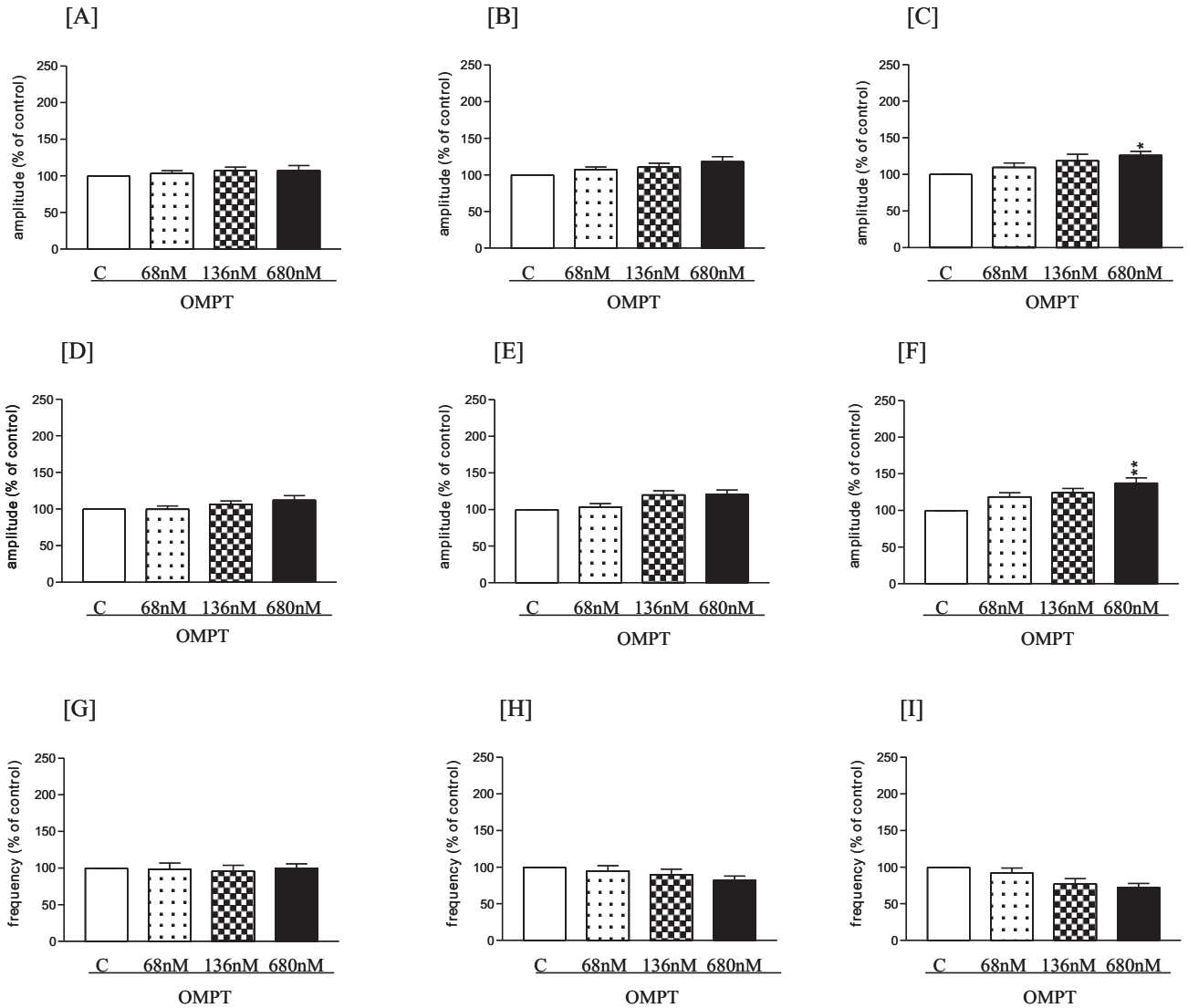

Fig. 4. Influence of 1-oleoyl-2-*O*-methyl-*rac*-glycerophosphothionate (OMPT) on the tension ([A],[B],[C]) amplitude ([D],[E],[F]) and frequency ([G],[H],[I]) of the contraction of the porcine myometrium strips of the control group ([A],[D],[G]), uterine horn without embryos ([B],[E],[H]) and with the embryos ([C],[F],[I]). Uterine strips were collected on days 12-14 of the estrous cycle (control group) or pregnancy (groups with embryos and without embryos). Values (mean ± SEM; n = 5 in each group) are expressed as a percentage of changes in the contractile activity before the treatment. * $P < 0.05$, ** $P < 0.01$, as compared to the contractile activity before the treatment (C).

developing embryos, L-α-LPA at all doses increased ($P < 0.05$ -0.001) the tension (Fig. 1C), ($P < 0.01$, ($P < 0.001$) amplitude (Fig. 1F), while the frequency of contractions was enhanced only at the highest concentration of the studied agent (Figs. 1I, 5A).

In the MYO strips L-α-LPA did not affect significantly ($P > 0.05$) all parameters examined in both the control and experimental group (Fig. 2).

Influence of OMPT on the contractile activity of uteri

In the ENDO/MYO uterine strips of the control group, OMPT did not change significantly ($P > 0.05$) all parameters examined (Fig. 3A,D,G). In the ENDO/MYO strips of the uterine horn without the developing embryos after OMPT administration, an increase in the contraction tension (at a dose of 680 nM; $P < 0.05$; Fig. 3B) and amplitude (at a doses of 136 and 680 nM; $P < 0.05$ and $P < 0.01$, respectively; Fig. 3E) was observed (Fig. 5D). However, the frequency of contractions after administration of the highest dose of OMPT was decreased ($P < 0.05$) (Fig. 1H). In the ENDO/MYO strips of the uterine horn with the embryos, OMPT increased ($P < 0.01$) the contraction tension at the highest dose (Fig. 1C), and amplitude at all doses examined (Fig. 1F), while the frequency of contractions at doses of 136 nM

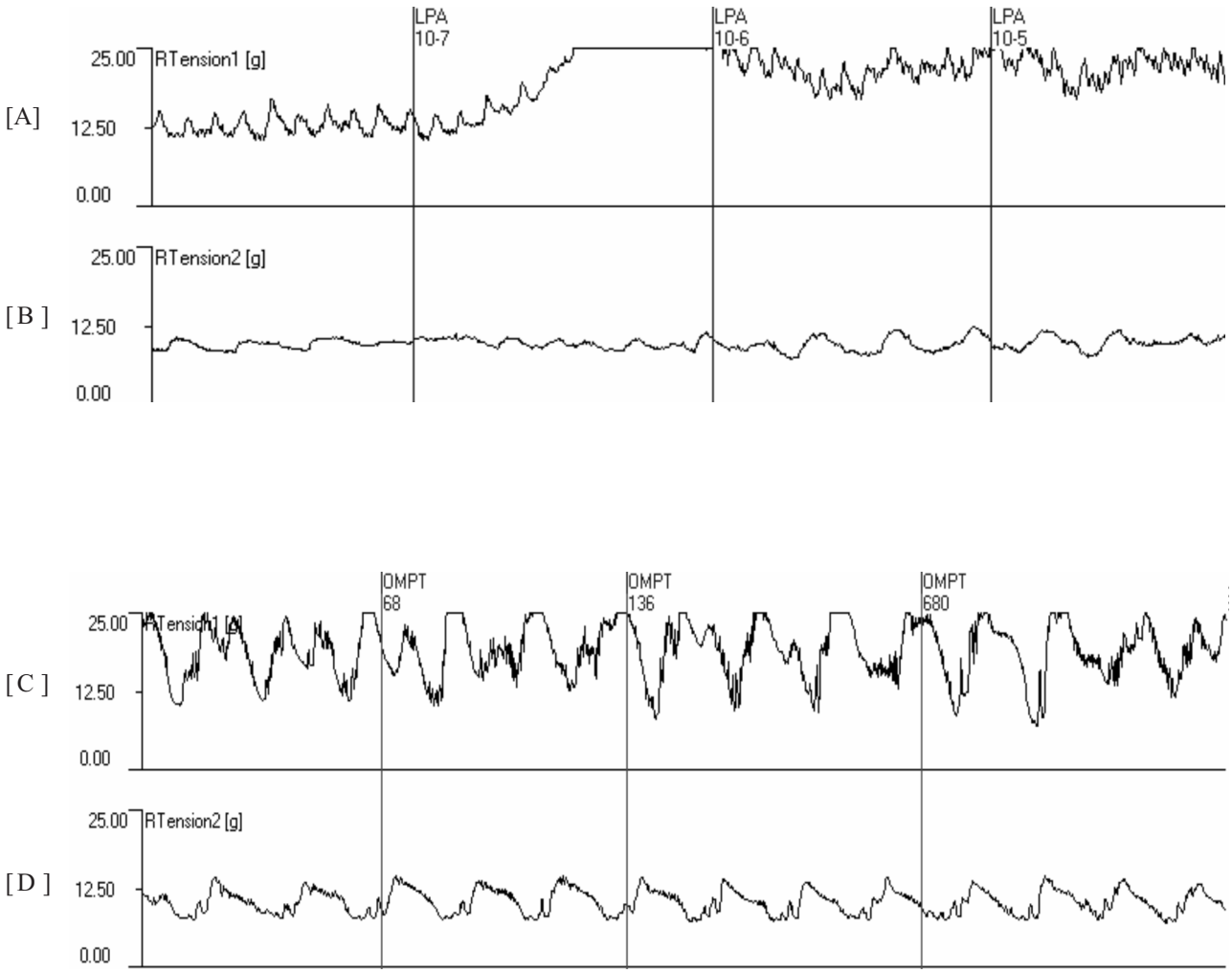

Fig. 5. Representative diagram showing the influence of oleoyl-sn-glycero-3-phosphate (L-α-LPA) and 1-oleoyl-2-*O*-methyl-*rac*-glycerophosphothionate (OMPT) on the contractile activity of the porcine endometrium/myometrium strips collected on days 12-14 of pregnancy. Each panel shows the contractile activity in the response to increasing doses of L-α-LPA (10^{-7} – 10^{-5}M) in the uterine horn with the embryos (Fig. 5A) and without embryos (Fig. 5B) and OMPT (68 nM; 136 nM and 680 nM) in the uterine horn with the embryos (Fig. 5C) and without embryos (Fig. 5D).

($P < 0.05$) and 680 nM ($P < 0.01$) (Fig. 3I) was decreased (Fig. 5C). In the MYO strips of the uterine horn with the developing embryos, OMPT administration at the highest dose caused an increase in the contraction tension ($P < 0.05$; Fig. 4C) and amplitude ($P < 0.01$; Fig. 4F) while the frequency of contractions was not affected.

Discussion

Since the first report that LPA induces cell proliferation and differentiation similar to that induced by growth factors via G protein-coupled receptors (Van Corven et al. 1989), many studies have shown that LPA participates in diverse physiological functions such as angiogenesis, neuronal and cardiovascular development, immunomodulation, smooth muscle contraction and relaxation, and tumorigenesis (Ishii et al. 2004). There is not much data about LPA function in the uterus but recent reports have suggested that LPA receptor signaling plays an important role in the embryo implantation process (Ye et al. 2005). In the studies conducted on mice (Ye et al. 2005, Hama et al. 2007) it was found that improper functioning of LPA in the uterus may result in delayed implantation, improper embryo spacing along the uterine horns and disturbed embryo development. In the present study we showed that L-α-LPA increased the contractile activity in the ENDO/MYO from the uterine horn with the developing embryos but did not affect the contractility in the uterine horn without the embryos. OMPT

increased the contractile activity in ENDO/MYO strips of both uterine horns with and without the embryos, and in MYO strips of the uterine horn with the embryos. It may suggest that activation of LPA_1 and LPA_2 is possible only in the presence of embryos, while OMPT acts rather through LPA_3 with the stronger effect in the uterus of pregnant pigs (regardless of the embryo presence in the uterine horn) comparing to the cyclic uterus. Our results are consistent with the previous study performed by Hama et al. (2007). They found that that selective agonist of LPA_3 (1-oleoyl LPA) caused contraction of smooth muscles of the pregnant mouse uterus. However, such effect was not observed in the uterus of mice lacking gene responsible for LPA_3 synthesis but with the preserved contraction activity confirmed by reaction to acetylocholine. Moreover, Kaminska et al. (2008) showed higher level of mRNA for LPA_3 gene in the porcine endometrial tissue on days 6-7 of pregnacy as comared to the cyclic uterus. Higher mRNA level was also found in the uterine horn containing embryos compared to the contralateral horn, where embryos did not develop. These results imply the important role of receptor LPA_3 during early pregnancy.

In conclusion, direct activation of LPA_1, LPA_2 i LPA_3 in the porcine myometrium during early pregnancy results in the enhanced contraction activity of smooth muscles. Furthermore, it was found that not only LPA_3 but also two other isoforms of the receptor, LPA_1 and LPA_2, participate in contractions of the uterus in pregnant pigs. The present data indicate the important role of LPA and its receptors in the induction of uterine contractions what is the main factor influencing embryo spacing.

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