Functional characterisation of an ovine oxytocin signal transduction pathway in oxytocin receptor cDNA transfected Cos 7 cells.

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Expression of the endometrial oxytocin receptor (OTR) between days 12-14 of the oestrous cycle initiates luteolysis and determines the length of the luteal phase in non-pregnant ruminants [1-4]. Inhibition of OTR synthesis during the fertile cycle is essential for early embryo survival during the maternal recognition of pregnancy [5]. Cloning and sequencing of a human myometrial OTR cDNA [6] has assigned the OTR to the G-protein-coupled receptor family characterised by seven transmembrane domains. Oxytocin (OT) activates phosphoinositide (PI)-specific phospholipase C (PLC) in ovine endometrial tissue leading to the generation of two potential second messengers, inositol 1,4,5-tris phosphate (IP_3) and diacylglycerol (DG) [7, 8]. Understanding the nature of the OTR coupling to this transducing mechanism and subsequent initiation of a signal cascade is important in terms of attributing biological significance to the generation of second messengers as mediators of oxytocin action.

We have used individual treatments with OT, a highly selective OT antagonist (OTA), pertussis toxin (PTX), non-specific G-protein stimulators (GTPyS, AIF-4) and a novel PLC inhibitor U73122 (inactive isomer U73343) to investigate the mechanism of receptor coupled G-protein-mediated regulation of PLC in OTR cDNA transfected Cos 7 cells.

We recently cloned a full length ovine OTR cDNA using a specific probe which was derived from oestrous endometrial mRNA and primers corresponding to the sixth and seventh transmembrane domains of a human OTR cDNA sequence [9]. The coding region of the cDNA was generated by PCR and subcloned into the SV40 major late promoter expression vector PSVL/J [10]. Cos 7 cells maintained at 37°C in DMEM + 10% FBS were seeded into 35mm dishes at 10^5 cells per dish (n=3 per treatment). The cells were transfected with the PSVL/J + OTR construct (negative control: PSVL/J-OTR, containing the insert in the opposite orientation) at 60% confluence using a modification of the DEAE-dextran method [11]. 24 hours post-transfection cells were labelled with [3H]inositol (1μCi/ml) and incubated overnight. Cells were washed twice with PBS at 37°C and the medium changed to DMEM + 2% FBS containing 10mM myo-inositol. For membrane impermeable treatments crude membranes were prepared by freeze/thawing twice and centrifugation. 4000g for 60 min at 4°C. A further 30 min incubation fresh DMEM + 2% FBS was added containing 10mM LiCl and the cells incubated for a further 30 min. Fresh LiCl supplemented medium was added and incubated with the following treatments: OT (10^{-9}M), OTA (10^{-9}M), OT+OTA, GTPyS (10^{-5}-10^{-9}M), AIF-4 (50nM), OT+PTX (200ng/ml), OT+U73122 (10^{-6}M) for up to 30 min. Incubations were terminated by the addition of perchloric acid and extracts neutralised with tripotassium orthophosphate. Accumulation of total inositol phosphates following each treatment was assayed by anion exchange [12].

OT in the presence of the expressed receptor stimulated PI turnover 4-fold in excess of residual endogenous activity (Fig.1A). This is comparable to that reported for endometrial tissue [8].

There was no response to OT in non-transfected cells or those transfected with the negative construct PSVL/J-OTR (Fig.1A). OTA did not increase PI turnover and competitively inhibited the OT-induced response (Fig.1A). The direct activation of PI turnover following treatment with the non-hydrolysable GTP analogue GTPyS and fluoride ions, active form AIF-4 (Fig.1A,B) suggested that the pathway was G-protein-coupled. Co-incubation of GTPyS with OT (10^{-9}M) shifted the threshold of activity from 10^{-5}M to 10^{-7}M and significantly increased the level of response (Fig.1B).

As in endometrial tissue [13] the OT signal transduction pathway was PTX insensitive (Fig.1A). This indicates that the functional G protein in the pathway is a member of the Gq class of the Gp family (p=phosphoinositose). Cos 7 cells are known to contain mRNA for two α-subunits (Gq and Gαo) of members of Gq [14]. In our system treatment with U73122 had no effect on OT-induced stimulated PI turnover (Fig.1A). This is consistent with the response in endometrial tissue [8] but contrary to its reported effect in disrupting receptor-coupled G-protein mediated regulation of PLC in polyomorphonuclear leukocytes [15].

This study clearly shows that Cos 7 cells have the required G protein to mediate OT-stimulated PI turnover and that the signalling pathway attributed to the expressed OTR resembles that of the in vivo receptor.


Figure 1. Accumulation of inositol phosphates in OTR cDNA transfected Cos 7 cells: (A) intact cells non-transfected + OT, transfected with PSVL/J-OTR + OT, transfected with PSVL/J + OTR and the following treatments: no OT, OT, OTA + OT, OT + PTX, OT + U73122, OT + U73343, AIF-4. (B) membranes following treatment with GTPyS alone or GTPyS + OT. Values shown are means ± S.E.M.; n=3.