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Calcium influx and release mechanism(s) in histamine-induced myometrial contraction in buffaloes

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ABSTRACT

The present study was undertaken to characterize the presence of histamine H1R using molecular biology tools and unravel the influx and release mechanism(s) involved in calcium signalling cascades in histamine-induced myometrial contraction in buffaloes. The presence of H1R mRNA transcript and immunoreactive membrane protein in buffalo myometrium was confirmed by RT-PCR and Western blot analysis. Further, histamine produced concentration-dependent (1 nM–10 μM) contraction in buffalo myometrium with a potency of 7.13 ± 0.11. When myometrial strips were pre-incubated either with Ca^{2+} free solution or with nifedipine, a L-type Ca^{2+} channel blocker, dose response curve (DRC) of histamine was significantly ($P < 0.05$) shifted towards right with decline in maximal contraction (E_{max}). Reduction in E_{max} of histamine in the presence of nifedipine (55.75 ± 3.10%) was significantly ($P < 0.001$) greater than that in the presence of ruthenium red (93.61 ± 3.43%), a blocker of IP3-gated and RyR-sensitive Ca^{2+} channels. Moreover, histamine produced only 26.87 ± 1.99% of the maximum contraction in the presence of both nifedipine and CPA (blocker of sarco-endoplasmic reticulum Ca^{2+}-ATPase). Interestingly, following concurrent exposure to U-73122 (a PL-C inhibitor) and nifedipine, the DRC of histamine was significantly ($P < 0.05$) shifted towards left with increase in maximal contraction (126.30 ± 3.36%). Our findings in buffalo uterus thus suggest that influx of extracellular calcium plays a major role in histamine-induced myometrial contraction, while release of intracellular calcium through calcium-release channels of sarcoplasmic reticulum has a minor role. A possible involvement of non-selective cation channels in histamine-induced myometrial contraction cannot be ruled out, and therefore requires further investigations.

1. Introduction

The presence of mast cells in the vicinity of uterine smooth muscle and placental tissue is well documented in mammals. Among the different mediators released following mast cell degranulation, histamine plays an important role in normal ovulation, blastocyst implantation, placental blood flow regulation and lactation besides regulating myometrial activity both in response to allergic or infectious stimuli and in the normal parturition process (Szelaq et al., 2002; Willets et al., 2008). Pregnancy-dependent alterations in histamine levels in blood and reproductive tissues have been documented in cattle and buffaloes (Matta et al., 1999, 2001). Histamine is reported to be involved in certain pathological processes such as pre-eclampsia, preterm delivery and

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birth of stillborn pups. Pre-treatment of mothers with H1 receptor antagonist, ketotifen, resulted in delivery of normal pups at term (Bytautiene et al., 2004). Accumulating evidences suggest that myometrial cells express histaminergic receptors and produce uterine contraction through activation of H1 receptors, whereas relaxation by H2 receptors (Rudolph et al., 1993; Bytautiene et al., 2002; Szelaq et al., 2002). H1 histamine receptors are G-protein-coupled receptors (GPCRs) and stimulate the generation of second messengers inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) which by increasing the cytosolic Ca^{2+} level induce myometrial contraction (Hill et al., 1997). Elucidation of signalling mechanisms involved in histamine-induced myometrial contraction may facilitate identification of newer and more selective novel therapeutic targets for addressing myometrial patho-physiological states in human beings and animals, especially in buffaloes. However, no information is available on the existence of H1 histaminergic receptors and their signalling mechanisms in buffalo myometrium. Thus, the present study was undertaken to unravel the calcium signalling cascades involved in histamine-induced myometrial contractions in non-pregnant buffaloes.

2. Materials and methods

2.1. Tissue source

For RNA isolation, uteri were collected from adult cyclic nondescript buffaloes immediately after slaughter from the local abattoir in 0.1% diethyl pyrocarbonate-treated autoclaved phosphate buffer saline (PBS) on ice and transported to laboratory in thermos flask. For protein extraction, uteri were collected in ice-cold PBS. After removing the adjacent tissues, uterine strips were quickly snap frozen in liquid nitrogen and stored in −80 °C until use.

For tension experiments, uteri along with ovaries were collected in oxygenated and chilled (4.0 ± 0.5 °C) Ringer-Locke solution (RLS) of following composition (mM/L): NaCl, 154; KCl, 5.6; $CaCl_2{\cdot}2H_2O$, 2.2; $NaHCO_3$, 6.0; D-Glucose, 5.5 and having pH of 7.4. Diestrous stage uteri were selected based on the well developed projected (crowned) corpus luteum on ovary, genitalia with closed cervix and thick mucus. Further, uteri were cut open to rule out the possibilities of early pregnancy.

2.2. RT-PCR analysis

Frozen uteri were thawed in TRI-zol® reagent (Ambion, Life Technologies) and total RNA was extracted employing guanidinium isothiocyanate–phenol–chloroform method and suspended in 15 μl of nuclease free water. The purity of the RNA was checked by A260/A280 ratio and quantified as 1 OD = 40 μg/ml. DNase-treated total RNA (4 μg) was then reverse transcribed by oligo $(dT)_{18}$ primer using a first strand cDNA synthesis kit (Thermo Scientific, USA). The resulting cDNA samples were amplified by PCR with gene specific oligonucleotide primer pairs. Gene sequence of histamine H1 receptor (H1R) in buffaloes is not reported in NCBI, thus primer sequences were designed from the information available for bovine (*Boss Taurus*, Acc. No. D10197.1 and NM_174083.3) using DNA STAR and Gene Tool software. Gene specific primers used in the present study to amplify H1R in buffalo uterus were F-5′TGTGCAGCAGCCCCTCAAGTAC3′ and R-5′GGAAGGACCCGTTGATGAGCTC3′. PCR was performed using the reaction mixture containing 10X Taq buffer with 25 mM $MgCl_2$ (2.5 μl), 10 mM dNTPs (0.5 μl), dream Taq (0.2 μl), cDNA (2 μl) and 20 pM of each primer (0.5 μl) in a final volume of 25 μl. The amplification condition consisted of pre-denaturation at 95 °C for 3 min followed by 32 cycles of denaturation at 95 °C for 30 s, annealing at 63 °C for 30 s and extension at 72 °C for 1 min, followed by final extension at 72 °C for 5 min. The PCR products were resolved by 1.5% agarose gel electrophoresis and the amplicon size was verified by simultaneously running a 100 bp DNA ladder (Fermentas, USA). The PCR product was then extracted from gel by the kit method (Qiagen) and subsequently cloned in a TA cloning vector pTZ57R/T using InsTA clone PCR cloning kit (Thermo Scientific, USA). Identity of the clone was confirmed by sequencing the cloned product from Department of Biochemistry, Delhi University, South Campus, New Delhi, India.

2.3. Uterine membrane preparation

The frozen uterine samples were thawed on ice and membrane proteins were isolated as described by Ducza et al. (2001). Briefly, samples were homogenized in ice-cold buffer composed of 10 mM Tris–HCl, 1 mM EDTA, 0.6 mM $MgCl_2$, and 0.25 M sucrose, pH 7.4. The suspension was then filtered on four layers of gauze and centrifuged at 40,000 × *g* for 20 min at 4 °C. The resulting pellet was then re-suspended in lysis buffer (50 mM Tris–HCl, pH 8.0, 0.5% deoxycholate, 1% Triton X-100, 0.1% SDS, 1% phenylmethylsulfonyl fluoride and 1 mg/ml aprotinin) and stored at −80 °C. Protein concentration was determined by Bradford method (1976).

2.4. Western Blot assay

Uterine membrane protein samples (40 μg per well) were diluted in Laemmli buffer and denatured for 3 min at 100 °C in boiling water bath. Proteins were separated on 12% SDS polyacrylamide gels using GeNei™ electrophoresis unit and subsequently electrically transferred onto a polyvinylidene difluoride (PVDF) membranes at 200 mA for 2 h using GeNei™ transfer apparatus containing transfer buffer (20 mM Tris, 192 mM glycine and 20% methanol). The membranes were then blocked for 1 h at room temperature in a blocking buffer containing 5% (w/v) skimmed milk powder in PBS (pH 7.4). After washing, the blots were incubated overnight at 4 °C with rabbit anti-histamine receptor 1 (H1R) polyclonal antibody (AB5652P; Chemicon, Millipore) diluted (1:200) in PBS containing 0.05% (v/v) Tween-20 (PBS-T). The blot was then washed and incubated for 1 h at room temperature with goat anti-rabbit IgG conjugated with horseradish peroxidase (AP187P; Chemicon, Millipore) at 1:500 dilution in PBS-T. After three washings of membrane with PBS-T for 30 min,