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Pharmacological characterization of store-operated calcium channels (SOCC) in myometrium of non-pregnant buffaloes (*Bubalus bubalis*)

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ABSTRACT

Present study unravels the existence and functional involvement of store-operated calcium channels in myometrium of non-pregnant buffaloes. Uteri along with ovaries were collected from nondescript adult cyclic buffaloes immediately after their slaughter from the local abattoir. Under a resting tension of 2 gm, effect of $CaCl_2$ in the absence and presence of different blockers/modulators of calcium regulatory pathways was recorded. $CaCl_2$ produced concentration-dependent contraction and the DRCs of $CaCl_2$ were significantly ($P < 0.05$) shifted to right in the presence of nifedipine (1 µM) + CPA (10 µM) and nifedipine (1 µM) + CPA (10 µM) + 2-APB (10 µM). After incubation of myometrial strips with nifedipine + CPA in Ca^{2+} free RLS (-Ca^{2+}), histamine was added to the tissue bath to allow the release of Ca^{2+} from SR while having already blocked the SERCA by CPA (10 µM) to prevent the Ca^{2+} reuptake into SR and nifedipine was used to prevent entry of Ca^{+2} from VDCC when calcium chloride was added and 2-aminoethoxydiphenyl borate (2-APB) (10 µM) was used as a non-specific blocker of store-operated calcium channels (SOCC). In the presence of nifedipine + CPA + 2-APB, calcium chloride produced contractile effect and the maximal contraction observed was only 0.62±0.14 g (n=6) which was significantly ($P<0.05$) lower compared to that of 1.20±0.10 g (n=6) in the presence of nifedipine + CPA in normal Ca^{2+} free RLS. This observation indicated that after depletion of Ca^{2+} from Sarcoplasmic reticular, SOCC got activated and in the presence of 2-APB, response was significantly reduced. Thus implying the functional involvement of store-operated calcium channels in myometrium of non-pregnant buffaloes.

Key words: 2-APB, Buffalo myometrium, Calcium chloride, Store-operated Ca^{2+} Channel

Calcium entry via voltage-gated calcium channels forms the predominant calcium entry pathway and it is involved in contraction in excitable cells, including myometrium. Evidence about the functional involvement of store-operated calcium entry (SOCE) and receptor-operated calcium entry (ROCE) in myometrial tissue and cells exists (Sanborn, 2007; Dalrymple *et al.* 2004, 2007; Noble *et al.* 2009). These pathways not only contribute to prolongation of the contraction events, but also to uterine smooth muscle growth and discrete calcium signals required to modulate gene expression throughout gestation and at term for onset of labor (Chin-Smith *et al.* 2014).

Agonist-induced increase in intracellular free Ca^{2+} concentration is an important component of the signaling pathways (Berridge *et al.* 2000) which increase the cytosolic free-Ca^{2+} in cells by release of Ca^{2+} from the intracellular stores and by influx of Ca^{2+} from the extracellular media into the cell. Recently, sarco-endoplasmic reticular calcium ATPase (SERCA) has been associated with depolarization in mouse myometrium (Gravina *et al.* 2010). Sarcoplasmic reticulum (SR) Ca^{2+} has a profound effect on intracellular Ca^{2+} signals (Shmygol and Wray, 2005) and decrease in SR Ca^{2+} load causes potentiation of Ca^{2+} transients and contractions after inhibition of SERCA by cyclopiazonic acid (CPA) (Taggart and Wray, 1998). In pregnant rat uterus, CPA is reported to inhibit the phasic contractions and transform the myometrial activity to tonic type and is associated with large increase in the baseline Ca^{2+} (Taggart and Wray, 1998). Presence of Stim-Orai proteins (Murtazina *et al.* 2011) and Trp homologues in human myometrium (Dalrymple *et al.* 2002) add evidence to the presence of SOCE in myometrium (Noble *et al.* 2009). We hypothesize that Ca^{2+} release from the SR activates SOCE via SOCC or calcium release-activated calcium (CRAC) channels or capacitative Ca^{2+} channels which causes depolarization of the uterine smooth muscle cells. The main objective of the present study was to investigate the effect of SERCA pump inhibition and to facilitate calcium depletion to activate the store-operated calcium channels to regulate Ca^{2+} signaling and contractions in buffalo myometrium.

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MATERIALS AND METHODS

Uteri along with the ovaries were collected from nondescript adult cyclic buffaloes immediately after slaughter from the local abattoir and transported to laboratory in chilled (4.0±0.5°C) Ringer-Locke solution (RLS) having pH of 7.4. Diestrous stage of the cycle was ascertained based on the well developed projected (crowned) corpus luteum on ovaries, genitalia with closed cervix and thick mucus. Myometrial strips were prepared and mounted in thermostatically controlled (37.0±0.5°C) organ bath (Ugo Basile, Italy) of 10 ml capacity containing RLS continuously aerated with carbogen (95% O_2 + 5% CO_2) under a resting tension of 2 g as per the method described earlier (Sharma *et al*. 2012). During the equilibration period of 2 hrs, bath fluid was changed after every 10 min.

Calcium chloride, nifedipine, 2-aminoethoxydiphenyl borate (2APB), Nickel chloride ($NiCl_2$) and cyclopiazonic acid (CPA) were procured from Sigma-Aldrich (USA). Except nifedipine (dissolved in ethanol), all other chemicals were dissolved in distilled water and stored at 4°C except CPA which was stored at –20°C. Further dilutions of the required concentrations were made in freshly prepared RLS on the day of use. The solvents used for preparation, at the concentrations finally used, did not influence myometrial activity. The myometrial strips were incubated with different antagonist/blockers for a period of 30 minutes. 'n' denotes the number of animals for each tension experiment.

Isometric tension in myometrial strips was recorded with the help of force transducer (Panlab, Spain) using Lab Chart Pro V6.1.3 software (Powerlab, AD Instruments; Australia). Effects of different blockers/antagonists on calcium chloride ($CaCl_2$) induced responses were calculated in relation to the maximal myometrial contraction (g tension) produced by $CaCl_2$ alone. The concentration–response curves were constructed by measuring the height/ average maxima using the Labchart pro v 6.1.3 software. EC_{50} and E_{max} values were determined by non-linear regression analysis using Graph Pad Prism 4.0 (Graph Pad, La jolla, USA) and pD_2 value (potency) was calculated as – log of $EC_{50.}$ Results are expressed as mean±SEM. Multiple mean values were analyzed using two-way ANOVA followed by Bonferroni post hoc test to compare between the different treatments while student's 't' test was used to compare between the two groups only and one-way ANOVA followed by Tukeys post-hoc tests for more than two groups.All the statistical analysis were done using Graph Pad Prism 4.0 (Graph Pad, La jolla, USA)

RESULTS AND DISCUSSION

Calcium chloride produced concentration-dependent contractile effect in Ca^{2+}-free RLS and the maximum contraction (1.98±0.36 g; n=8) was achieved at 10^{-2} M bath concentration as shown in Fig. 1A. In the presence of nifedipine, a L-type calcium channel blocker, calcium chloride produced contraction similar to that of calcium chloride alone. Compared to control, the dose response curve (DRC) of $CaCl_2$ was significantly ($P<0.05$) shifted towards right as shown in Fig. 1B and there was significant decrease in E_{max} value (Table 1). These observations suggest that in Ca^{2+}-free RLS or even after blockade of VDCC by nifedipine, some myometrial contraction was present which suggests that probably some other channels are available or become operative for producing calcium chloride-induced myometrial contraction after blockade of L-type VDCC. Possible involvement of calcium influx through receptor-operated Ca^{2+} channels (Andersson, 1995) and/or calcium release activated Ca^{2+}-channels in buffalo myometrium cannot be ruled out (Berridge, 1995). But it requires further studies.

To unravel the involvement of SOCC and/or calcium release activated Ca^{2+}-channels in calcium–induced

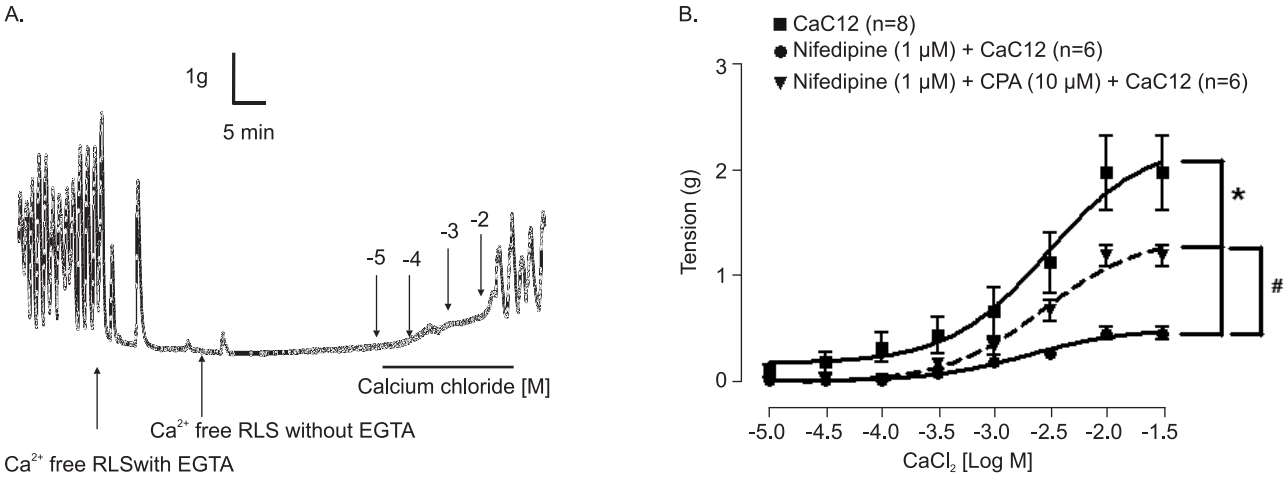

Fig. 1. Representative physiograph recordings (1A) showing the effect of calcium chloride alone on myometrial spontaneity in Ca^{2+} free RLS. Cumulative concentration response curves (1B) of calcium chloride alone and in the presence of nifedipine + $CaCl_2$ and nifedipine + CPA+ $CaCl_2$ on myometrial strip of non-pregnant buffaloes. Vertical bars represent SEM. Data were analyzed by two-way ANOVA followed by Bonferroni post-hoc tests. *$P<0.05$ vs $CaCl_2$ alone v/s nifedipine + calcium chloride or nifedipine + CPA + calcium chloride, # $P<0.05$ vs nifedipine + $CaCl_2$.v/s nifedipine + CPA + calcium chloride.

Table 1. Comparative potency (pD_2) and efficacy (E_{max}) of calcium chloride on uteri of non pregnant buffaloes in the presence of Ca^{2+}-free Ringer Locke Solution.

Treatment	pD_2	E_{max} (g)
$CaCl_2$ alone (n= 8)	2.52 ± 0.23	1.98 ± 0.36
Nifedipine + $CaCl_2$ (n= 6)	2.69 ± 0.15	0.46 ± 0.06*
Nifedipine + CPA + $CaCl_2$ (n= 6)	2.55 ± 0.11	1.20 ± 0.10*#
Nifedipine + CPA + 2APB + $CaCl_2$ (n= 6)	2.71 ± 0.23	0.62 ± 0.14*#ä

contraction in Ca^{2+}-free RLS, myometrial strips were concurrently incubated with nifedipine and CPA in Ca^{2+} free RLS (-Ca^{2+}) and histamine was used to allow the release of Ca^{2+} from SR while simultaneously blocking SERCA by CPA (10 µM) to prevent Ca^{2+} reuptake into SR and nifedipine to prevent entry of Ca^{+2} from VDCC when calcium chloride was added. Calcium chloride produced contractile effect and the maximal contraction observed was 1.20±0.10 g (n=6) which was significantly (P<0.05) lower compared to that of 1.98±0.36 g (n=8) of calcium chloride alone in Ca^{2+} free RLS and significantly (P<0.05) higher than in the presence of nifedipine (0.46±0.06 g (n=5) in Ca^{2+} free RLS as evident from the Fig. 1B and Table 1. The DRC of calcium chloride was further significantly (P<0.05) shifted towards left compared to in the presence of nifedipine alone in Ca^{2+} free RLS as evident from Fig. 1B. Thus, suggesting that following depletion of intracellular Ca^{2+} by histamine, there is activation of SOCCs which are involved in inducing significantly higher $CaCl_2$-induced contraction compared to that in the presence of nifedipine alone. Agonist-induced intracellular Ca^{2+} transients are biphasic; initial transient rise in Ca^{2+} represents the sarcoplasmic Ca^{2+} release while the later sustained Ca^{2+} response represents predominantly the Ca^{2+} influx (Putney, 2004; Putney *et al.* 2004). One of the mechanisms of extracellular Ca^{2+} influx is store-operated Ca^{2+} entry and store-operated Ca^{2+} entry mediated by a transient receptor potential ion channels (TRPC) (Putney *et al.* 2004; Van Rossum *et al.* 2004). In this process, depletion of intracellular Ca^{2+} stores leads to activation of channels located in the plasma membrane that are permeable to Ca^{2+} (van Rossum *et al.* 2004).

To confirm the influx through SOCC, 2-APB (10 µM) was used as a non-specific SOCC blocker. In the presence of nifedipine + CPA + 2-APB, calcium chloride produced contractile effect and the maximal contraction observed was only 0.62±0.14 g (n=6) which was significantly (P<0.05) lower compared to that of 1.20±0.10 g (n=6) in the presence of nifedipine + CPA in Ca^{2+} free RLS as shown in Fig. 2A-B. A.

These data suggested that after depletion of Ca^{2+} from

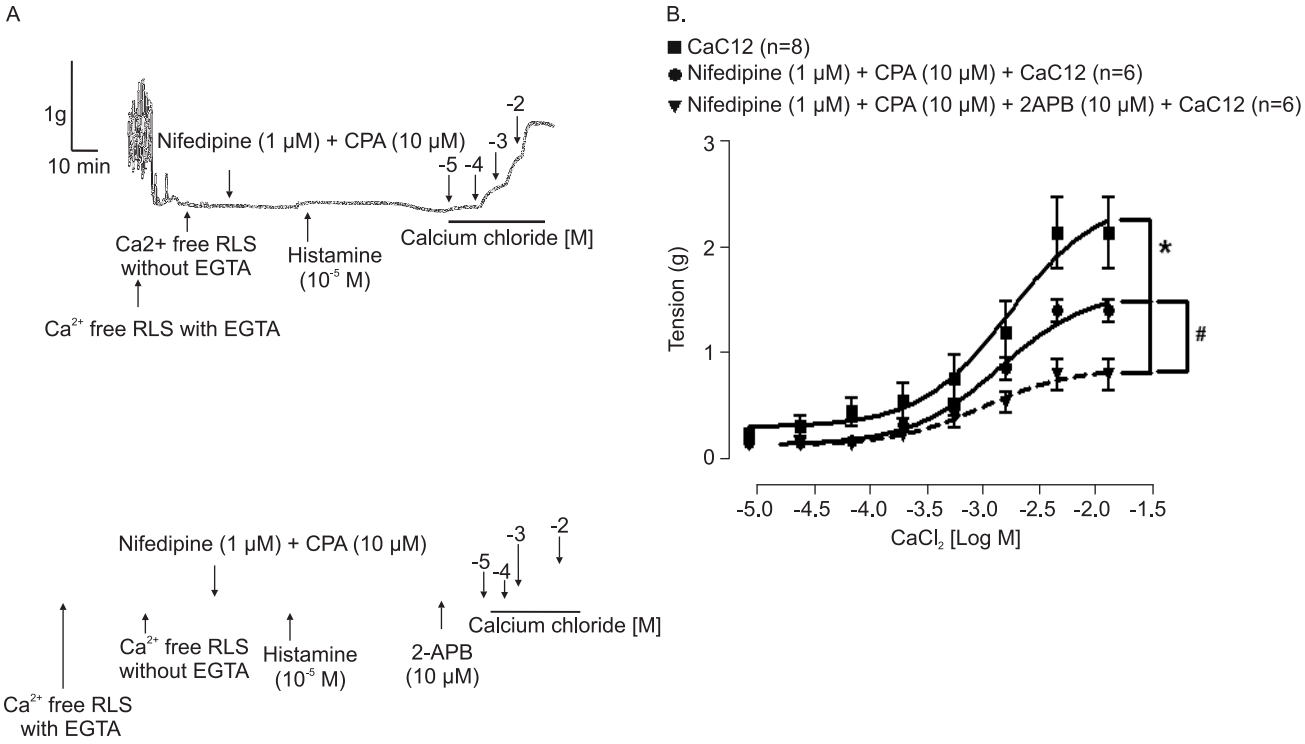

Fig. 2. Representative physiograph recordings (2A) showing the effect of calcium chloride in the presence of nifedipine + CPA and nifedipine + CPA+ 2-APB on myometrial spontaneity in Ca^{2+} free RLS. Cumulative concentration response curves (2B) of calcium chloride alone and in the presence of nifedipine + CPA (10 µM) and in the presence of 2-APB (10 µM) + nifedipine + CPA (10 µM) on myometrial contraction in non-pregnant buffalo uterus. Vertical bars represent SEM. Data were analyzed by two-way ANOVA followed by Bonferroni post-hoc tests.

*P<0.05 calcium chloride alone v/s nifedipine + CPA + calcium chloride or nifedipine + CPA + 2-APB + calcium chloride, #P<0.05 nifedipine + CPA + calcium chloride v/s nifedipine + CPA + 2-APB + calcium chloride.

SR, calcium chloride induced effect was regulated by store-operated calcium channels. Existence of store–operated calcium channels has been reported in myometrial cells of humans (Murtazina *et al.* 2011; Shlykov *et al.* 2003; Monga *et al.* 1999) and animals (Dalrymple *et al.* 2002).

In conclusion, functional involvement of store-operated calcium channels in myometrium of non-pregnant buffaloes and after depletion of Ca^{2+} from Sarcoplasmic reticular, SOCC got activated. Signalling mechanism in buffaloes cannot be ruled out; therefore, further studies on these aspects are warranted.

Conflicy of Interest

None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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