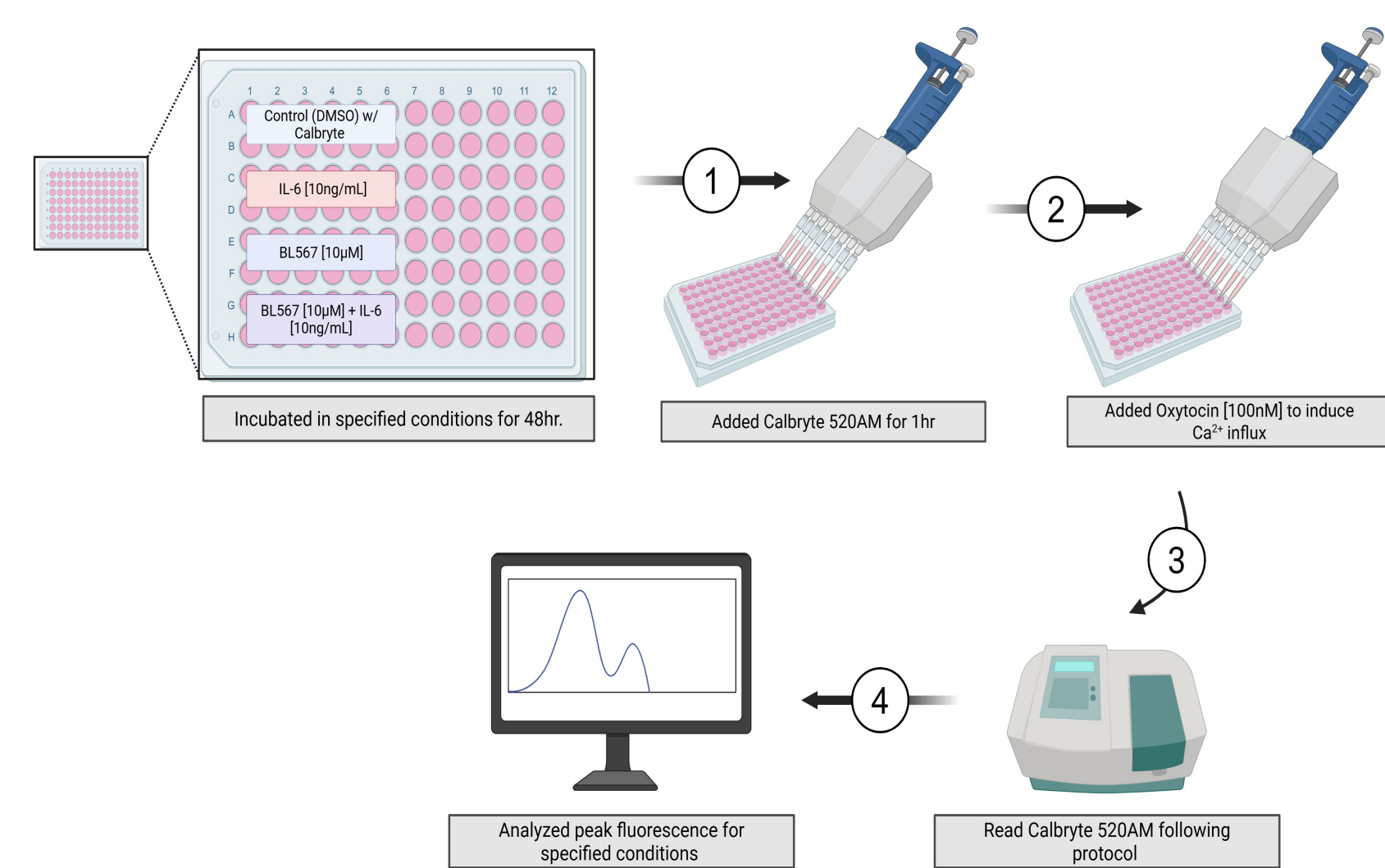


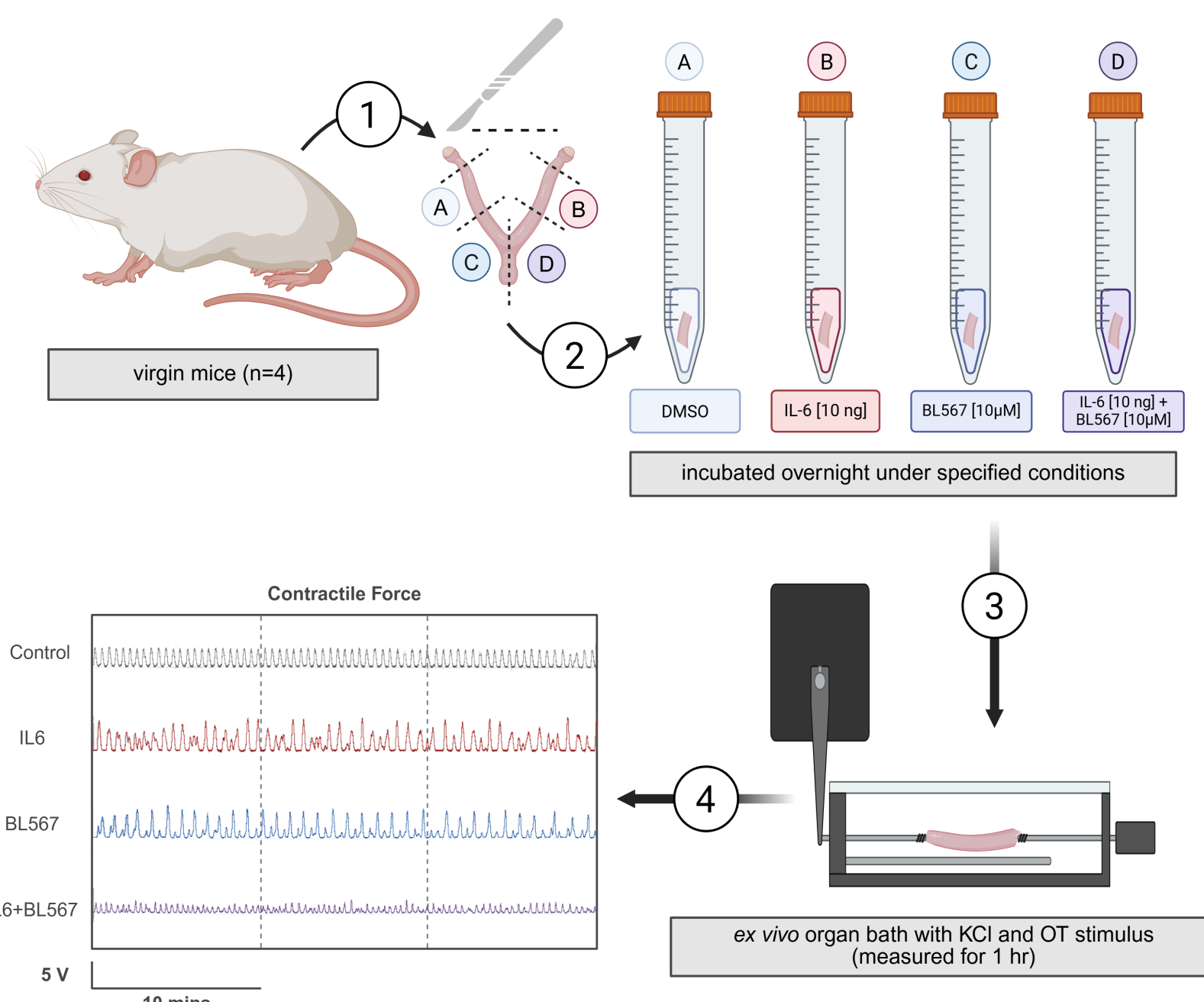
ABSTRACT

Approximately 10% of births in the United States are preterm (before 37 weeks' gestation). Tocolytics are therapies designed to halt early labor; however, there are no FDA-approved tocolytics. Oxytocin receptor (OXTR) is known to increase calcium influx following stimulation by oxytocin, resulting in calcium-induced calcium-release, spurring uterine contractions in preterm and term parturition. Previous findings demonstrate that the pro-inflammatory cytokine, IL-6, increases OXTR expression in uterine smooth muscle. Cyclotriazadisulfonamide (CADA) compounds have been shown to downmodulate membrane proteins (such as OXTR). We hypothesize that IL-6 sensitizes uterine smooth muscle and promotes OXTR regulation of calcium influx, leading to enhanced contractility, while our CADA compound, BL567, reduces OXTR expression, thereby reducing contractile force. This research may further the development of novel tocolytic therapies.

METHODS



Graphical abstract of Intracellular Calcium Signaling Assay. Ran in triplicate on pregnant human uterine smooth muscle cells (phUSMCs) and analyzed using Prism.



Graphical abstract of incubation and organ bath on mice uteri in specified conditions. Analyzed using LabChart Reader and Prism.

RESULTS

BL567 Decreases OXTR Expression

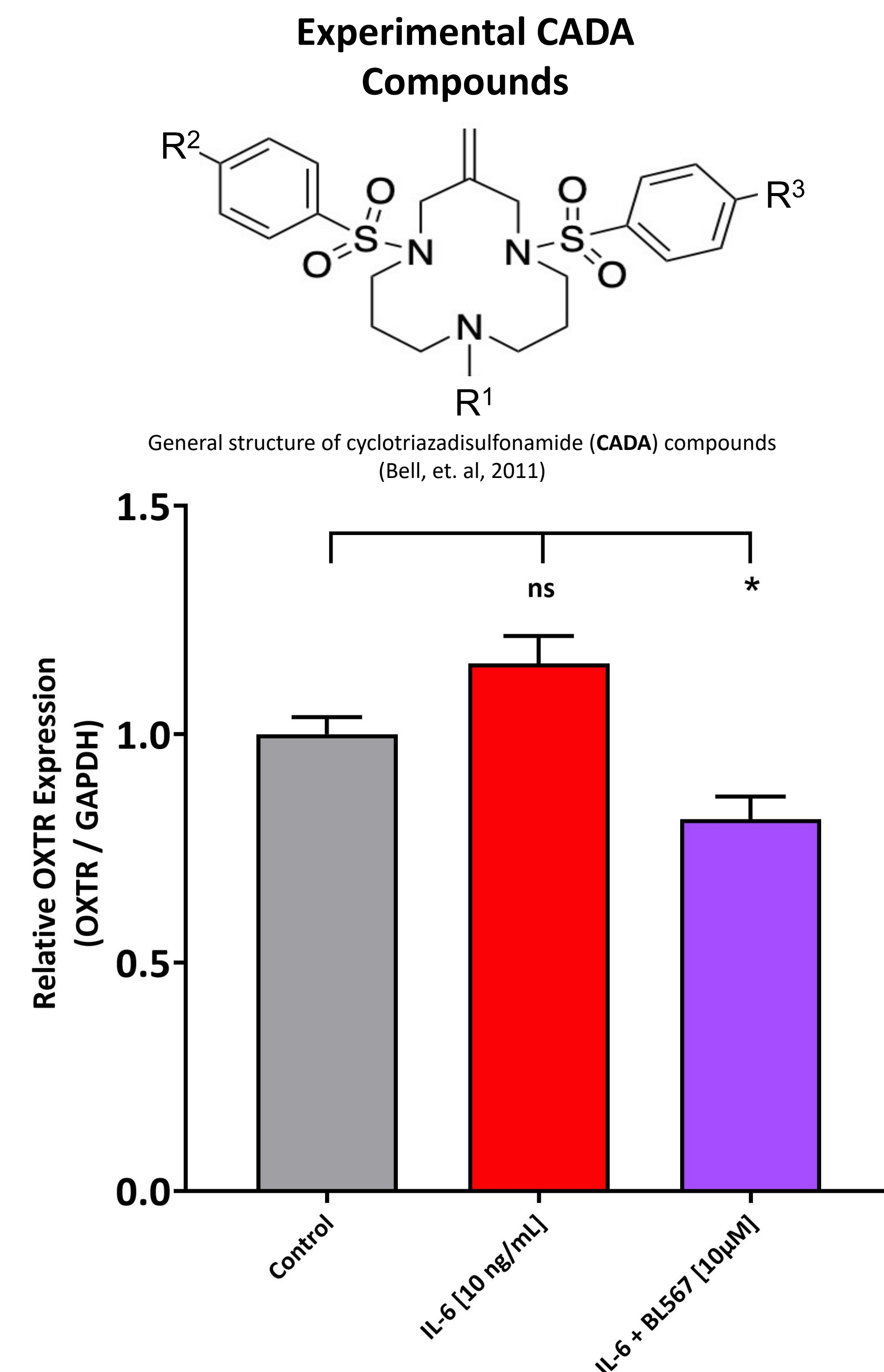


Figure 1. (Top) Representative chemical structure of parent CADA compound. BL567 stems from this structure. (Bottom) phUSMC were cultured in 100 mm dishes (n=3) and treated with vehicle control (DMSO), 10 µM BL567, or 10 µM BL567 ± IL-6 (10 ng/mL) for 48 hours. After incubation, cells were lysed and analyzed by Western blotting. Post-hoc analysis using an unpaired t-test revealed that the presence of BL567 significantly mitigated the IL-6-induced increase in oxytocin receptor (OXTR) expression (p = 0.0396).

BL567 Decreases Ca²⁺ influx in Uterine Smooth Muscle Cells

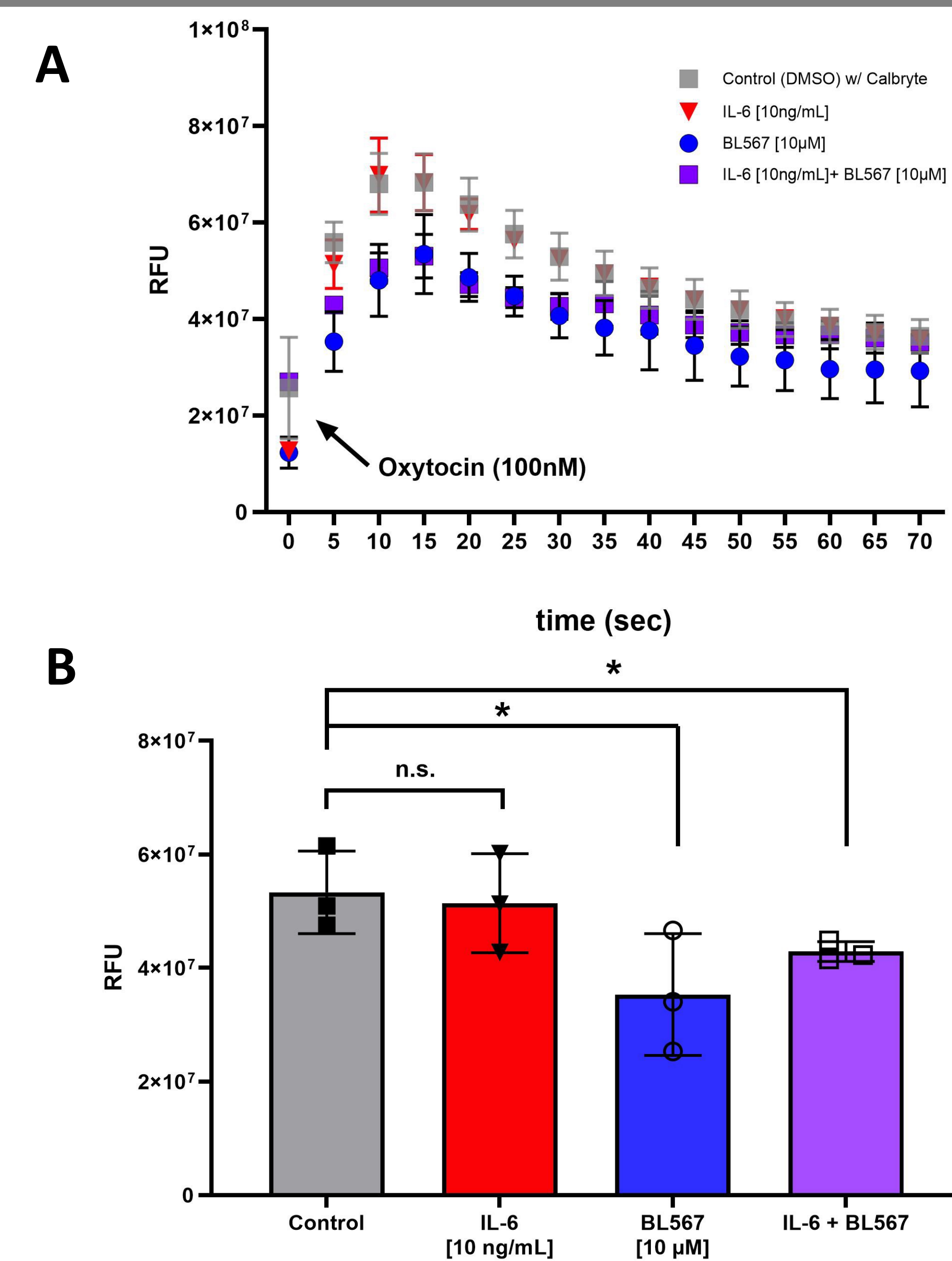


Figure 2. phUSMC (n=3; passage 5-8) were grown to confluency in a flat bottom, black, 96 well plate. Cells were growth arrested for 48 hours followed with treatment of IL-6 [10ng/mL], BL567 [10 µM] or a combination of both. Following treatment, cells were loaded with Calbryte® 520AM for 1 hour, rinsed, then challenged with 100 nM oxytocin (OT) to determine the effect of the treatments on OXTR mediated handling. (A) graph representing relative calcium influx following OT exposure over 60 seconds. (B) calcium handling was not significantly different in IL-6 treated cells (P=0.3924) one tail t-test, however; in BL567 and IL-6+BL567 treated cells calcium influx was significantly lower compared to control (P=0.0386), (P=0.0366) one tail t-test, respectively.

BL567 imparts Negative Inotropic Effects on Uterine Smooth Muscle Tissue

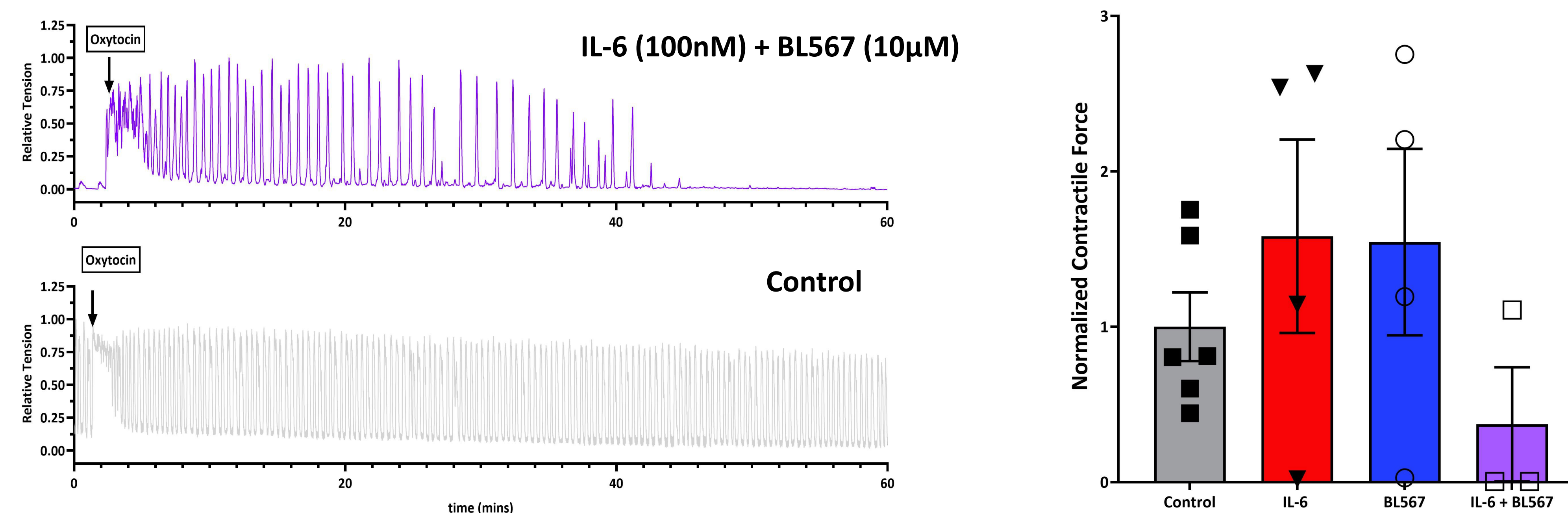


Figure 3. Uteri from virgin mice (n=4) were dissected into segments (Figure 2) and incubated overnight in oxygenated Krebs Complete Buffer under control or treatment conditions. Tissue strips were placed in an ex vivo organ bath system, tensed to 1g, and challenged with 60 mM KCl followed by oxytocin [8 nM] stimulation to assess contractility. Tissues that failed to produce a contractile response to OT were excluded. Relative tension was recorded for 1- hour post-stimulation. (Top Left) Tissue incubated in IL-6 + BL567 saw a decrease in contractile force to near zero. (Bottom Left) Control samples maintained consistent activity (Right) Relative contractile activity was compared across all conditions.

DISCUSSION AND CONCLUSIONS

Here we sought to investigate the tocolytic properties of BL567 by examining its effects on intracellular calcium (Ca²⁺) levels in pregnant human uterine smooth muscle cells (phUSMC) and its inotropic effects on mice uteri:

- Earlier data by Rauk *et al*, 2001 determined IL-6 increases OXTR expression. We did not observe this with our preliminary data; however, our data suggest a significant decrease in OXTR activity when exposed to either BL567 or IL-6+ BL567.
- In phUSMC, IL-6 did not alter Ca²⁺ influx however, BL567 and IL-6 + BL567 did significantly decrease compared to control.
- In an ex vivo organ bath, IL-6 demonstrated a trending increase, relative to control. The combination of IL-6+ BL567 exhibited weaker contractile force relative to control.

This data indicates the therapeutic potential of BL567 to ameliorate the contractile effects of sterile inflammation on uterine smooth muscle cells. In future experiments, sample size will be increased. In addition, IL-6 will be incubated for 5 hours rather than 48 hours to determine peak OXTR expression and subsequent contractile activity.

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