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Lignocaine augments the in-vitro uterine contractions involving NO-guanylyl cyclase dependent mechanisms

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ABSTRACT

Aims: Lignocaine is used during intrapartum and postpartum period but there are conflicting reports regarding the effect of lignocaine on uterine contractility. Therefore, this study was undertaken to delineate the effect of lignocaine on uterine contractility and the underlying mechanisms.

Main methods: The in vitro contractions were recorded from the uterine segments obtained from adult rats (in estrous phase) and also from human myometrial tissue. Effect of lignocaine on spontaneous uterine contractions was recorded in the absence or presence of antagonists. Effect of sodium nitroprusside (SNP, NO donor) on uterine contractility was assessed. The NO_2^- was assayed (indicator of NO activity) from the supernatant after exposing the myometrial tissue to lignocaine in the absence or the presence of L-NAME or hemoglobin.

Key findings: Lignocaine (100 μM) increased the amplitude of uterine contractions by 75% with no alterations in frequency. Similar magnitude of increase was seen with human myometrial tissue also. The spontaneous activities were absent in Ca^{2+}-free or in nifedipine (10 μM) containing medium. Heparin (IP_3 blocker, 10 IU/ml), but not the indomethacin (10 μM) blocked the lignocaine-induced augmentation. L-NAME (NOS inhibitor, 10 μM) or methylene blue (guanylyl cyclase inhibitor, 100 μM) partially blocked the lignocaine-induced augmentation. SNP (30 μM) increased the amplitude of spontaneous uterine contractions. Lignocaine increased the NO_2^- content (indicator of NO activity) of uterine tissue and the increase was blocked by L-NAME or hemoglobin.

Significance: Present observations indicate that lignocaine augments the amplitude of uterine contractions via Ca^{2+}-dependent mechanisms involving NO-G cyclase-dependent mechanisms.

1. Introduction

Local anesthetics are being widely used in obstetrical and gynecological interventions as an analgesic/anesthetic agent. Local anesthetics are known to alter the contractility of uterus [1–5] and thus may alter the outcome of intervention. However, the literature is not consistent for the effect of local anesthetic on uterine contractility. In studies elsewhere, different local anesthetics (bupivacaine, levobupivacaine, ropivacaine, tetracaine) including lignocaine have shown to decrease the uterine contractility in both pregnant and non-pregnant rats [4,5]. Yet in another report, bupivacaine and ropivacaine decreased the myometrial contractility, while another class of drug, mepivacaine significantly increased the uterine contractility [1]. In another study, lignocaine increased the myometrial contractions in vitro [2]. However in human myometrial strips, lignociane decreased the uterine contractility [3]. Thus the effect of local anesthetics on myometrial contractility varies with the class and type of local anesthetic used. Moreover the effect of local anesthetic on uterine contractility also depends upon hormonal status as estrogen increases the myometrial contractility while progesterone decreases it [6]. Effect of lignocaine on uterine tissue also varies with different concentrations as suggested elsewhere [7]. Thus, a clear data showing the effect of local anesthetic is not available. Considering these points, we performed the experiments on rats in estrous phase only and examined the effect of lignocaine on uterine contractility. Also we delineated the mechanism of actions of lignocaine on uterine contractility. Further, we also performed experiments with human myometrium, to confirm the actions of lignocaine seen in rat myometrium, to have a translatory value.

2. Materials and methods

2.1. Animals

Animal experiments were performed after obtaining approval by the

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Institutional Ethical Committee of Banaras Hindu University for conducting animal experiments. Adult albino Charles Foster female rats weighing 150–225 g were used in this study. All the animals were housed in animal room (25 ± 0.5 °C, having 12:12 h light:dark cycle) with ad libitum supply of food (Pashu Aahar, Varanasi) and water. Animals were allowed to adapt to the animal room for 3–4 days. Animals showing the estrous phase were selected for the experiments.

2.2. Identification of estrous phase

Estrous phase was detected in rats by the examination of vaginal smear. Vaginal fluid was aspirated by Pasteur pipette after instilling of normal saline (0.2–0.3 ml) in vaginal cavity. Then the aspirate was collected and smeared on glass slide and dried. The smear was stained with methylene blue (0.5 g/dl) for 15 min, then washed gently with running tap water and dried for microscopic examination (high power). The estrous phase was identified by the presence of non-nucleated cornified cells in the smear.

2.3. Dissection and isolation of tissue

The animals showing estrous phase were killed by cervical dislocation. The lower abdomen was opened quickly by midline incision. Uterus was identified and the two cornua were dissected free from the surrounding tissue. The excised uterine tissue was placed in a petridish containing Tyrode solution, bubbled with 100% oxygen. Small segment (10–15 mm length) of uterus was cut and secured to a glass tube (with an 'N' type of bending) via a silk thread (0).

2.4. Placement of tissue and recording

The uterine tissue with glass tube was transferred to 20 ml Dales' bath, pre-filled with Tyrode solution at 35 ± 1 °C, bubbled continuously with 100% O_2. The tissue was connected to force-displacement transducer via a thread. An initial tension of 1 g was given. The preparation was allowed to stabilize for 30 min. The spontaneous contractions were recorded on a chart recorder (Powerlab Data acquisition of ADInstruments with the help of Lab Chart 5 software).

2.5. Human tissue experiments

Some experiments were also performed on human myometrium. The human ethical clearance was obtained from the Institutional Ethical Committee of Banaras Hindu University, Varanasi, India for human studies. The informed written consent was obtained from non-pregnant women undergoing hysterectomy for gynecological causes (like fibroid, prolapsed uterus, menorrhagia etc.) in Sir Sunderlal Hospital, Varanasi. A small piece of uterine tissue (which appeared normal) from the gynecology operation theatre was dissected and placed in wide mouth bottle containing oxygenated Tyrode solution. The bottle was then transported in an ice chamber to the Department of Physiology for recording contractions. Myometrial strip (2–3 mm wide and 10–15 mm long) was dissected out from uterus, endometrium was removed then it was connected to a glass tissue holder. The method for tissue preparation, recording setup and other parameters were similar to rat experiments as described earlier.

2.6. Drugs and solutions

Lignocaine was obtained from Trikaa pharmaceuticals Ltd., Dehradun, India. N-omega-nitro-L-arginine methyl ester (L-NAME), indomethacin and sodium nitroprusside (SNP; nitric oxide donor) were obtained from Sigma Aldrich Chemicals, Milwaukee, WI, USA. Heparin was obtained from Biological E. Limited, Haridwar, Uttarakhand. Methylene blue was obtained from Glaxo Smithkline Pharmaceuticals Ltd., Mumbai, India. Hemoglobin (Hb) was obtained from Loba Chemie Pvt. Ltd. Mumbai, India. Chemicals for the Griess reagent were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. All other chemicals were of analytical grade. Tyrode solution had the following composition (mM) (NaCl, 137; KCl, 3.7; $MgCl_2.6H_2O$, 0.05; $CaCl_2.2H_2O$, 1.2; $NaH_2PO_4.2H_2O$, 0.3; $NaHCO_3$, 11.9 and glucose, 5.0; pH, 7.3). Stock solution of nifedipine was prepared in ethanol and all other chemicals were prepared in distilled water. The solutions were then refrigerated and thawed just before use.

The dose of lignocaine (100 μM) on uterus was chosen from the earlier report where they have shown the dose-response of lignocaine [3,7]. The dose of indomethacin (PG synthase inhibitor) was 10 μM and was taken from Hargrove [8] and Phillippe et al. [9]. The doses of L-NAME (NOS inhibitor; 10 μM), hemoglobin (Hb; nitric oxide quencher, 100 μM), methylene blue (guanylyl cyclase inhibitor; 100 μM), SNP (NO donor, 30 μM), nifedipine (L type of calcium channel blocker, 10 μM) and heparin (non-specific IP_3 blocker; 10 IU/ml.) were selected from our earlier studies [10–16].

2.7. Experimental protocol

The experiments were divided into six main groups. In group I to group V, the experiments were performed on rat uterus and in group VI, the experiments were performed on human uterus.

In group-I (n = 8), after stabilization for 30 min, spontaneous uterine contractions were recorded and were considered as initial. Then 0.1 ml of Tyrode solution (as mentioned above) was added to organ bath and recordings were made after 15 min. Further, the tissue was exposed to lignocaine (100 μM) and recordings were made after 15 min.

In group-II (n = 8), after initial recordings, tissue was exposed to Ca^{2+} free medium/nifedipine (10 μM) containing medium and recordings were made after 15 min. Further lignocaine (100 μM) was added in the Ca^{2+}-free medium/nifedipine containing medium and recordings were made as before.

In group-III (n = 16), after the initial recordings, tissue was exposed to various antagonists (indomethacin, 10 μM; L-NAME, 10 μM; heparin, 10 IU/ml, methylene blue, 100 μM) for 15–20 min, then the recordings were made. Further, lignocaine (100 μM) was added in the organ bath and recordings were made after 15 min.

In group-IV (n = 8), after initial recordings, tissue was exposed to sodium nitroprusside (SNP; 30 μM) and recordings were made after 20 min.

In group-V (n = 24), the nitrite (NO_2^-) content of the supernatant fluid was assayed after exposing the uterine tissue to 0.1 ml Tyrode solution or lignocaine (100 μM) in the absence or presence of L-NAME (10 μM) and Hb (100 μM) for 30 min. Only one antagonist was tested in a single preparation (n = 6 for each group).

In group-VI (n = 8), effect of lignocaine on human myometrial tissue was recorded. After stabilization for 30 min, spontaneous uterine contractions were recorded and were considered as initial. Then 0.1 ml of Tyrode solution was added to organ bath and recordings were made after 15 min. Further tissue was exposed to lignocaine (100 μM) and recordings were made after 15 min.

2.8. NO_2^- estimation

The NO_2^- was estimated by Griess reagent, as described earlier [17]. Briefly the uterine horns were dissected out, cut into small pieces and transferred to tubes containing 20 ml Tyrode solution bubbled with 100% O_2 at 35.0 ± 0.5 °C. In the control group, only Tyrode solution was added into the tube. The other tubes were pre-treated with equal volume of Tyrode solution/L-NAME (100 μM)/Hb (100 μM) for 20 min and subsequently lignocaine (100 μM) was added. After 20 min, the uterine tissue was homogenized and centrifuged separately. The NO_2^- content of the supernatant was assayed using Griess reagent by the methods described earlier [18]. Briefly, 100 μl of the supernatant was added to each of the marked wells in 96-well microplates. Equal volume