

Evaluation of Relaxant Effect of Levromakalim on Oxytocin Induced Contraction of Isolated Rat Uterus

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ABSTRACT

Objective: To observe the relaxant effect of Levromakalim (a Potassium Channel Opener) on the Oxytocin induced contractions of the non-pregnant, isolated rat uterus. **Method:** This study was carried out in the Department of Pharmacology, Shaikh Zayed, Post Graduate Medical Institute, Lahore. The Female non-pregnant albino rats (Sprague dawley strain), weighing approximately 150 to 200g were included in the study. The living animals were injected with Diethylstilbestrol (0.1 mg/kg), intramuscularly, 24 hrs before the experiments to make the uterus more sensitive to the effect of Oxytocin. The animals were then sacrificed, the uterus of each animal was dissected out and the isolated uterus was placed in the tissue organ bath, containing DeJalon's solution which was aerated with 100% oxygen and the temperature of the system was maintained at 32°C. First, the normal activity of the isolated uterus was recorded and then the effect of Oxytocin (contractions) was observed. The dose of Oxytocin (1-5ng) was required to produce sub-maximal contractions of the uterus. Later on Levromakalim (a potassium channel opener) relaxant effect was observed. The dose of Levromakalim (1-5µg) was required to reduce 50% uterine contractions produced by Oxytocin. **Results:** The concentration (dose) of Oxytocin required to produced the sub-maximal contractions of the rat uterus was (Mean±SEM) 4.360±0.644ng. The dose of Levromakalim required to decrease 50% contractions of the uterus induced by Oxytocin was (Mean±SEM) 3.041±0.364µg. **Conclusion:** The study proved that Levromakalim (a Potassium Channel Opener) is a relaxant of the rat uterus and it can be clinically used in Dysmenorrhea and Pre-mature labour in humans.

Key Words: Levromakalim, Oxytocin, Rat Uterus, Potassium Channels.

INTRODUCTION

Levromakalim (a Potassium Channel opener or Lactivator) is a novel smooth muscle relaxant and prototype of Potassium Channel activator drugs. It acts by opening potassium channels in the plasma membrane of the cell so that the membrane potential of the smooth muscle is moved to a more negative potential close to the potassium equilibrium. This potential is more negative than the threshold potential at which calcium channels open, reducing calcium influx therefore, Levromakalim relaxes or inhibits excitation of smooth muscles¹.

Mode of action of Levromakalim

Levromakalim → Potassium Channel opening → enhanced potassium efflux → hyperpolarization of the cell membrane → reduced calcium entry / release → smooth muscle relaxation.

The sequence of events from Potassium Channel opening to smooth muscle relaxation demonstrates a link between Potassium Channel opening, hyper polarization and reduction in both Ca^{+2} influx and the release of Ca^{+2} from intracellular store².

Types of Potassium Channels

Recent electrophysiological studies and the

use of new pharmacological agents have shown that potassium channels are present on cells of several types including nerves, secretory cells, skeletal and smooth muscle cells³.

All cells in the body are likely to have potassium channels and they may have more than one type⁴.

1. **Voltage Sensitive Potassium Channels:** Depolarization of excitable cells leads to opening of a potassium channel, which brings about repolarization or hyper polarization of the cell. These channels therefore function to inhibit excitatory processes. Voltage sensitive Potassium Channels are well described in nerves and skeletal muscle and several types have been described with different conductances and timing of opening³.
2. **Calcium Activated Channels:** Open when the concentration of intracellular calcium ions rises (either from entry of calcium through voltage dependent or receptor operated calcium channels or release from intracellular stores). Several types of channels, with high, intermediate, or low conductance have been recognized. These may be blocked selectively by different pharmacological agents. Calcium activated Potassium Channels have been described in airway smooth muscle⁵.
3. **Receptor Operated Potassium Channels:** Activation of certain surface receptors such as muscarinic, and adenosine receptors, may open these channels. Some receptors may be linked directly with the Potassium Channel, whereas others may depend on intermediary G Proteins³.
4. **ATP Controlled Potassium Channels:** These channels may respond to reduce intracellular concentration of ATP. The best-described example is in pancreatic islet β cells⁶.

Pharmacological Effects of Levromakalim

Cromakalim relaxes rat uterine smooth muscles⁷.

Cromakalim relaxes uterine muscles *in vitro*⁸.

The uterine relaxant activity of Cromakalim has been confirmed following I.V. administration to conscious non-pregnant and day 18 pregnant rats⁹.

Levromakalim and other Potassium Channel openers may have therapeutic potential in preterm labour or dysmenorrhoea^{1,10}.

Cromakalim inhibited the spontaneous activity of the myometrium and vaso pressin induced contractions of myometrium and intramyometrial arteries. The combined effect on the uterus and intrauterine vasculature would suggest a potential use of Cromakalim for the treatment of dysmenorrhoea¹¹.

Levromakalim (Cromakalim) suppressed the spasm evoked by Potassium Chloride¹².

Levromakalim beside the smooth muscle of the uterus also relaxes various other smooth muscles of the body for example blood vessels¹³, effect on the heart¹⁴, airway smooth muscle¹⁵, Urinary bladder smooth muscle^{16,17}.

Due to the above mentioned pharmacological effects on various smooth muscles, the drug has the potential for therapeutic uses in hypertension¹⁸, Angina pectoris¹⁹, Bronchial asthma²⁰, Bladder Hyperactivity¹⁷, Gastro Intestinal motility disturbances such as Irritable Bowel Syndrome².

OBJECTIVE

To observe the relaxant effect of Cromakalim (Levromakalim) on Oxyt-ocin induced contractions in the isolated rat uterus.

MATERIALS AND METHODS

The female albino non-pregnant rats of Sprague dawley strain weighing about 150-200g were used in the present study. Dejalon's physiological salt solution was used for rat uterus preparation²¹. Levromakalim solution was prepared in 70% ethanol: distilled water²². Oxytocin solution was prepared in distilled water⁷. Diethylstilbestrol solution prepared in ethanol²³.

Levromakalim was obtained from SK & B (U.K.) Diethylstilbestrol from Sigma Chemical Co. Oxytocin from Sandoz and Geofman Pharmaceutical Laboratories.

METHOD

The rat uterus preparation

The rats were injected with diethylstilbestrol intramuscularly 0.1 mg/kg 24 hours before the experiments. The animals were sacrificed and the abdomen opened. The two horns of the uterus were dissected out and transferred to a petridish containing Dejalon's solution. The two horns were separated and freed from fat, each horn was cut and opened longitudinally so that the preparation became like a sheet of muscles instead of a narrow tube. A thread was attached to each end of the tissue and the preparation mounted in isolated tissue organ bath containing Dejalon's solution which was aerated with oxygen (100%) and temperature maintained at 32 °C. One end of the tissue was attached to a fixed pin in the organ bath and the other end with force displacement transducer by means of threads. Transducer connected to a kymograph (recorder). The preparation took about 30 minutes to settle down before regular responses were obtained²¹. First normal activity of isolated uterus preparation was recorded and then effect of Oxytocin was observed by taking different concentrations of Oxytocin solution till the maximum contraction achieved, then the concentration of Oxytocin which produced sub maximal response was found. After this Levromakalim relaxant effect was studied by using different concentrations of Levromakalim solution and that concentration was found, which reduced 50% uterine contractions produced by Oxytocin.

Statistical Analysis

Results are carried out by using Mean, Standard deviation, Standard error of the mean of data and by applying paired 't' test.

RESULTS

The relaxant effect of Levromakalim was observed on Oxytocin induced contractions of the rat uterus.

The dose of Oxytocin, (Mean \pm SEM) 4.360 \pm 0.644ng was required to produce the sub-maximal contractions of the rat uterus (Table-1).

Levromakalim dose (Mean \pm SEM) 3.041 \pm 0.364

μ g was required to reduce 50% contraction of rat uterus produced by Oxytocin (Table-2).

Table 1: Oxytocin dose and sub-maximal contraction (Mean \pm SEM; n = 25)

Oxytocin Dose (ng)	Response (mm)
4.360 \pm 0.644	7.720 \pm 0.323

Table 2: Levromakalim dose and inhibitory (relaxant) response on oxytocin induced contraction (Mean \pm SEM; n = 25)

Levromakalim Dose (μ g)	Response (mm)
3.041 \pm 0.364	3.833 \pm 0.173

DISCUSSION

The present study was undertaken to investigate the relaxant effect of Levromakalim (Potassium Channel opener) on Oxytocin-induced contraction of non-pregnant isolated rat uterus. Levromakalim reduces the contraction produced by Oxytocin. These observations are in tune with the previous studies^{7,9,24,25}.

CONCLUSION

This study proved Levromakalim (a Potassium Channel opener) is a novel relaxant of uterine smooth muscle in albino rats and this effect is due to opening of potassium channels present in smooth muscle of uterus, and this smooth muscle relaxant effect of Levromakalim can be employed in clinical conditions such as premature labour (tocolytic agent) and dysmenorrhoea in humans.

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