

## INVOLVEMENT OF K<sup>+</sup> CHANNELS IN THE RELAXANT RESPONSES TO VARIOUS AGONISTS IN ESTROGEN PRIMED RAT UTERUS

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**Abstract:** The present investigation was undertaken to study the effects of K<sup>+</sup> channel openers in the relaxant responses to various agonists in estrogen primed rat uterus. Adrenaline and isoprenaline produced a dose-dependent relaxation in the estrogen primed rat uterus. The relaxant responses were found to be significantly potentiated when the preparations were exposed to PSS devoid of calcium. The responses to isoprenaline were found to be greater in the preparations depolarized with 40 mM KCl instead of 80 mM KCl. KCl failed to produce any contractile effect in the presence of D-600. Further, the addition of D-600 completely relaxed the KCl depolarized rat uterus. Pinacidil and cromakalim failed to relax 80 mM KCl depolarized rat uterus. However, they produced dose-dependent relaxation in the preparations depolarized with 40 mM KCl. The relaxant responses to pinacidil and cromakalim were competitively blocked by procaine. However, they were not altered by either propranolol or cimetidine. The relaxant responses to isoprenaline and histamine were found to be potentiated by pinacidil and cromakalim. These results indicate that in rat uterus in addition to adenylate cyclase c-AMP, potassium channels are also involved in the relaxant responses to isoprenaline and histamine.

**Key words :** cromakalim                      pinacidil                      rat uterus

### INTRODUCTION

Ca<sup>2+</sup> ions present in the vicinity of contractile protein determine the degree of activation of smooth muscle cell. Depolarized preparations provide a new way of looking at the action of drugs on smooth muscle. They provide a means of studying the interrelations between drugs and calcium in the processes leading to contraction and relaxation (1). Recently, various K<sup>+</sup> channels linked to Ca<sup>2+</sup> channels have been identified in various smooth muscles which can specifically be opened by a relatively newer class of agents that include pinacidil and cromakalim. These agents hyperpolarize the membrane and close voltage sensitive calcium channels and thereby reduce the influx of calcium (2, 3, 4). In the light of above facts the present investigation was undertaken to study the

role of Ca<sup>2+</sup> ions and effects of K<sup>+</sup> channel openers on the relaxant responses to various agonists in estrogen primed rat uterus.

### METHODS

Young Albino virgin female rats of Wistar strain weighing 200-250 g were treated with diethylstilbesterol (100 µg/100 g, i.p.) 24 hrs before they were sacrificed by a sharp blow to the head and cutting the neck blood vessels. The abdominal wall was immediately opened and two uterine horns were quickly dissected out and mounted in organ baths containing De Jalon's solution maintained at 37 ± 1°C. The composition of De Jalon's solution mM was: NaCl, 112.0; CaCl<sub>2</sub>, 0.25, KCl, 4.69; glucose 2.68 and NaHCO<sub>3</sub> 5.95. The preparation was allowed to stabilize for 30 min. During this time bathing solution was changed every 10 min. The

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responses to various drugs were recorded on polygraph (Polyrite, INCO, India). Force displacement transducer was adjusted to exert a basal tension of 1 g.

After stabilization for 30 min, the uterine horns were exposed to the graded doses of KCl ( $8.0 \times 10^{-3}$  to  $8.0 \times 10^{-2}$  M). The maximum contraction was achieved with  $8.0 \times 10^{-2}$  M KCl. Further experiments were carried out using 40 and 80 mM KCl.

In the first set of experiments, the depolarized uterine horns were exposed to graded concentration of isoprenaline ( $4.0 \times 10^{-8}$  M to  $1.21 \times 10^{-4}$  M) and adrenaline ( $3.0 \times 10^{-8}$  M to  $9.0 \times 10^{-5}$  M) added in a cumulative manner. The contact time for each agonist was 30 sec. After eliciting control dose-dependent relaxation responses of above agonists, the normal PSS was replaced by calcium free PSS or D-600 ( $1.0 \times 10^{-5}$  M) was added to organ bath and the preparations were allowed to stabilize for 15 min. Preparations were depolarized again with KCl (80 mM) and relaxant responses to all the agonists were recorded using this medium.

In second set of experiments, the preparations were depolarized with 40 mM and 80 mM KCl as described before. Pinacidil, ( $4.1 \times 10^{-9}$  M to  $1.0 \times 10^{-7}$  M) or cromakalim ( $3.5 \times 10^{-10}$  M to  $1.0 \times 10^{-7}$  M) were added in cumulative manner to record the relaxation. The responses to pinacidil and cromakalim were recorded again similarly in the presence of cimetidine ( $1.0 \times 10^{-6}$  M), propranolol ( $1.0 \times 10^{-6}$  M) or procaine ( $1.0 \times 10^{-6}$  M).

In another set of experiments, after depolarizing the preparations with 80 mM KCl, isoprenaline or histamine were added in cumulative manner. The dose dependent relaxation was re-elicited in presence of potassium channel openers.

Drugs used were: Adrenaline bitartrate, Isoprenaline hydrochloride, Histamine acid phosphate, Propranolol hydrochloride and Procaine hydrochloride (Sigma, USA) Cromakalim (Beecham Lab, U.K.), Pinacidil monohydrate (Leo Pharm., Denmark), Diethylstilbestrol (Allenburry's India), Cimetidine hydrochloride (Cadila Lab., India).

## RESULTS

Adrenaline (Fig.1a) and isoprenaline (Fig.1b) produced a dose dependent relaxation of estrogen primed

rat uterus depolarised with 80 mM KCl. The contractions by KCl were also obtained in the preparations bathed in PSS devoid of calcium chloride. However, it was not observed in presence of D-600. Addition of D-600 produced complete relaxation of KCl depolarized preparations. The relaxation produced by adrenaline and isoprenaline was faster in the preparations bathed with calcium free PSS. The responses to both the agonists were potentiated in the preparations bathed with calcium free PSS.

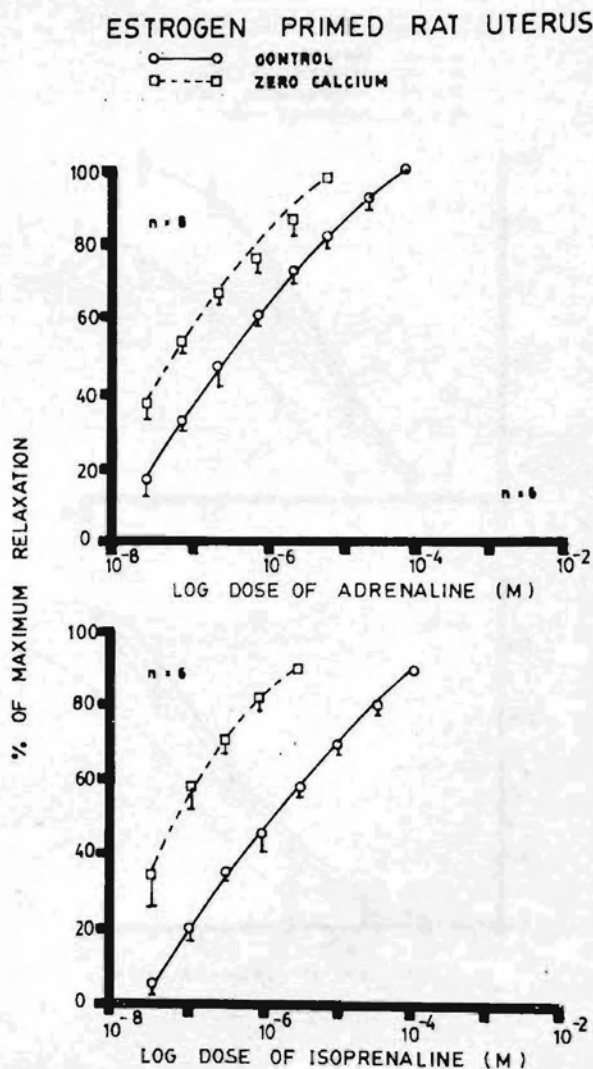


Fig. 1: Effects of adrenaline and isoprenaline on estrogen primed rat uterus bathed in normal and calcium free (zero calcium) PSS. Each point and the bar depict mean  $\pm$  SEM of 6-8 experiments.

Pinacidil and cromakalim failed to relax 80 mM KCl depolarized rat uterus. However, they produced a dose-dependent relaxation in the preparations depolarized with 40 mM KCl (Fig. 2b). The relaxant responses produced by pinacidil (Fig. 2a) and cromakalim (Fig. 2b) were competitively blocked by procaine.

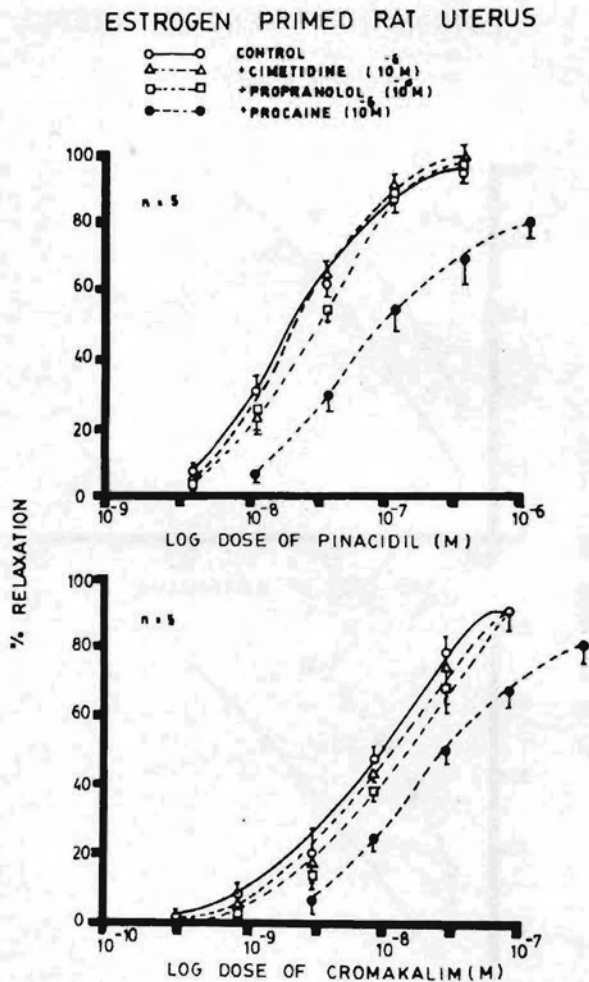


Fig. 2: Effects of pinacidil and cromakalim and their interaction with cimetidine ( $1.0 \times 10^{-6}$ M), and propranolol ( $1.0 \times 10^{-6}$ M) and procaine ( $1.0 \times 10^{-6}$ ) on estrogen primed rat uterus. Each point and the bar depict mean  $\pm$  SEM of 5 experiments.

However, they were not altered by either propranolol or cimetidine. Isoprenaline produced dose-dependent relaxation of estrogen primed rat uterus depolarized with 80 mM KCl. It also produced dose-dependent relaxation when the preparations were depolarized with 40 mM KCl. The relaxation by isoprenaline in the preparations depolarized with 40 mM KCl was significantly greater as compared to that obtained in the preparations depolarized with 80 mM KCl (Fig. 3a).

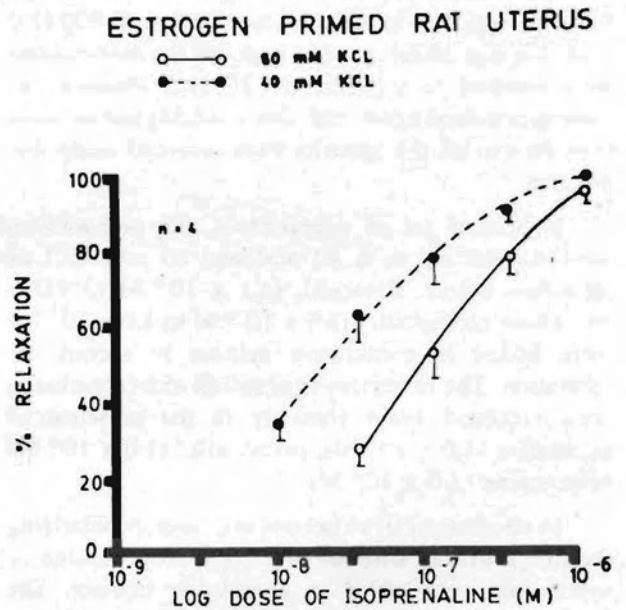


Fig. 3: Effects of isoprenaline on estrogen primed rat uterus when depolarized with 80 mM KCL. Each point and the bar depict mean  $\pm$  SEM of 4 experiments.

The responses to isoprenaline were further potentiated by pinacidil and cromakalim (Fig. 4a). Identical results were obtained with histamine. The relaxant effect of histamine was found to be potentiated by pinacidil and cromakalim significantly (Fig. 4b).

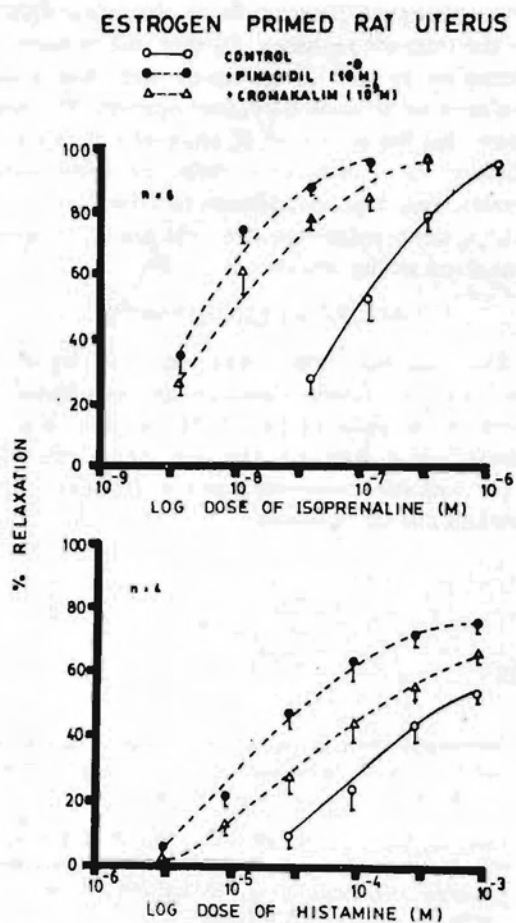


Fig. 4: Effects of isoprenaline and histamine and their interaction with pinacidil (1.0 × 10<sup>-9</sup>M), and cromakalim (1.0 × 10<sup>-9</sup>M) on estrogen primed rat uterus. Each point and the bar depict mean ± SEM of 4-6 experiments.

**DISCUSSION**

In the estrogen primed rat uterus, it was found that the preparation rapidly lost its responsiveness to KCl in Ca<sup>2+</sup> free solution. There is a decrease in the response to KCl on repeated stimulation in Ca<sup>2+</sup> free solution. This observation suggests the absence of a large store of sequestered Ca<sup>2+</sup> which can be mobilized by KCl. D-600 causes complete relaxation of KCl depolarized preparation, indicating that the muscle contraction produced by KCl in the Ca<sup>2+</sup> free PSS involves Ca<sup>2+</sup> loosely bound to cell membrane. This store is depleted during rapid stimulation but can be replenished from external medium if re-exposed to a calcium containing PSS. In the depolarized rat uterus,

adrenaline and isoprenaline produced a dose-dependent relaxation, which was found to be significantly potentiated when the preparations were exposed to the PSS devoid of CaCl<sub>2</sub>. The responses to adrenaline and isoprenaline are mediated through beta-adrenoceptors (5). Histamine also produced a dose-dependent relaxation of estrogen primed rat uterus. This relaxation is mediated through the stimulation of H<sub>2</sub> receptors, which in turn causes the release of catecholamines, to produce the effect through beta-adrenoceptors (6).

Previous data reported from our laboratory show that the responses to histamine were found to be potentiated in the rat uterus depolarized with 40 mM KCl instead of with 80 mM KCl (6). This potentiation may be due to reduced uptake of released noradrenaline (6). While we confirmed these findings, it was found that the relaxant effects of isoprenaline were also potentiated when the preparations were depolarized with 40 mM KCl. This observation suggests that some common mechanism other than the catecholamine uptake, may be involved in rat uterus.

The second messenger hypothesis (7) has been extended to indicate that cyclic AMP may be the medium for the relaxing effect of beta adrenergic agonists in smooth muscle (8, 9). This concept was subsequently referred to and expanded to propose the ratio of cAMP/cGMP as the controlling factor in the tissue responses (10). However, more recently, it has been shown that the changes in cyclic nucleotide levels can be dissociated from the changes in the contractility (11, 12). The dissociation between isoprenaline induced relaxation and the changes in cyclic AMP levels in uterine smooth muscle has been reported, concluding that cyclic AMP could not be the only determinant of inhibition of uterine contractility (13). In the present studies on rat uterus, it was found that relaxation caused by isoprenaline and adrenaline occurs more rapidly in the Ca<sup>2+</sup> free PSS as compared to that in normal PSS. When an explanation is sought for these observations, possibility may be that the relaxations produced by isoprenaline and adrenaline in depolarized muscle involve either an elimination of Ca<sup>2+</sup> into extracellular space or a shift of Ca<sup>2+</sup> intracellular to the sarcoplasmic reticulum. In normal PSS, high concentration of extracellular Ca<sup>2+</sup> relative to the presumably low free intracellular Ca<sup>2+</sup> will tend to oppose the extrusion of Ca<sup>2+</sup> and might interfere with the isoprenaline induced relaxation.

Cromakalim and pinacidil have been reported to relax guinea pig trachealis (14) guinea pig taenia caeci, rat vascular smooth muscle (3, 4) and rat portal vein (2). In present investigation, we have also observed that cromakalim and pinacidil produced a dose-dependent relaxation of KCl depolarized (40 mM) rat uterus. However, they failed to relax the uterus depolarized with 80 mM KCl. Our observation that potassium channel openers inhibit contractions to low but not high concentration of KCl is in agreement with the results obtained in other tissues like vascular, tracheal and gastrointestinal tract (14, 15). The action of potassium channel openers appears to be different from the action of calcium entry blockers which can inhibit contractions produced by all the concentrations of KCl. The relaxant effect of K<sup>+</sup> channel openers was significantly inhibited by procaine, a potassium channel

blocker. However, propranolol or cimetidine failed to alter the relaxant responses. Further, the responses to isoprenaline as well as histamine were found to be potentiated by potassium channel openers. The results suggest that the action of K channel openers is not mediated via beta-adrenoceptors or histaminergic receptors, and that in addition to adenylate cyclase cAMP system, potassium channels are also likely to be involved in the relaxation of rat.

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