Evaluation of tocolytic efficacy of selective beta\textsubscript{2} adrenoceptor agonists on buffalo uterus

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Present study was conducted on prostaglandin F\textsubscript{2alpha} (PGF\textsubscript{2a}), oxytocin, (OT), potassium chloride (KCl) and barium chloride (BaCl\textsubscript{2}) pre-contracted perimetal uterine strips of dioestrous and pregnant buffaloes to evaluate the tocolytic efficacy of selective beta\textsubscript{2} adrenoceptor agonists-albuterol (salbutamol) and terbutaline. Cumulative concentration-response curves of both the beta\textsubscript{2} adrenoceptor agonists were constructed and the mean effective concentration (EC\textsubscript{50}) values determined and compared statistically. Based on the comparative EC\textsubscript{50} values in relaxing the pre-contracted uterine strips with different spasmogens, the rank order potency of albuterol was found to be—PGF\textsubscript{2a} > BaCl\textsubscript{2} > OT > KCl on uterine strips from dioestrous animals, while OT > BaCl\textsubscript{2} > PGF\textsubscript{2a} > KCl on the uterine strips of pregnant buffaloes. The rank order potency of terbutaline on uterine strips from dioestrous stage animals was—BaCl\textsubscript{2} > OT > KCl > PGF\textsubscript{2a}, while BaCl\textsubscript{2} > PGF\textsubscript{2a} > KCl > OT on uterine tissues of pregnant animals. Thus, irrespective of the state of uterus, whether gravid or non-gravid, KCl-depolarized uterine tissues required comparatively higher concentrations of albuterol or terbutaline to produce tocolytic effect. High concentrations of K\textsuperscript{+} in biophase may have interfered with the beta\textsubscript{2} adrenoceptor agonists-induced outward K\textsuperscript{+} current and hyperpolarization. From the results of present study, it was evident that selective beta\textsubscript{2} adrenergic agonists had good tocolytic efficacy on the uterus of buffaloes. Further, indirectly the possibility of existence and activation of K\textsubscript{Ca} channels by selective beta\textsubscript{2} adrenoceptor agonists in mediating tocolysis of buffalo myometrium cannot be ruled out, however, detailed studies using specific K\textsubscript{Ca} channel blockers are required for characterizing the nature of such channels in buffalo uterus.

Keywords: beta\textsubscript{2} adrenoceptor agonists, Albuterol, Terbutaline, Buffaloes, Tocolytics.

Beta\textsubscript{2} adrenoceptor agonists have been reported to exhibit tocolytic effect in different species of animals including buffaloes\textsuperscript{1} and goats\textsuperscript{2}. Selective beta\textsubscript{2} adrenergic receptor agonists-induced myometrium relaxation has been postulated to be due to hyperpolarization\textsuperscript{3}. However, the pharmacodynamics of beta\textsubscript{2} adrenoceptor agonists-induced tocolysis of buffalo myometrium is yet to be elucidated. Therefore, the present investigation was undertaken to evaluate the tocolytic efficacy of selective beta\textsubscript{2} adrenoceptor agonists on isolated uterine strips of pregnant and dioestrous stage buffaloes pre-contracted with different spasmogens.

Materials and Methods
The non-gravid and gravid uteri along with the ovaries of non-descript breed buffaloes were collected in chilled Ringer-Locke solution in thermos flask from the local abattoir of Mathura. Immediately, the material was transported to the laboratory. Stage of the estrous cycle was assessed on the basis of thorough examination of the genital tract including ovaries. Pregnancy was confirmed based on the presence of foetus in the uterus and the corpus luteum on the ovary of the epilateral side. The stage of pregnancy was determined following the formula proposed by Soliman\textsuperscript{4} by measuring the curved crown versus rump length. Only, the uterine tissues from dioestrous and pregnant buffaloes were selected for detailed tocolytic studies using selective beta\textsubscript{2} adrenergic receptor agonists.

A piece of uterus was cut longitudinally from the mid-cornual region. Perimetal strips of about 0.5 × 3.0 cm prepared by carefully removing the endo- and myometrial tissues. The tissue-strips were mounted in an organ bath containing continuously aerated Ringer-Locke solution (10 ml) having a pH of 7.4 and maintained at 37°C±0.5°C. The tissues were allowed to equilibrate under a constant tension of 2g for 1 to 1.5 hr. During the equilibration period, the perfusion fluid was changed every 15 min. The responses of tissues to various chemicals/agents were recorded on a kymograph assembly with the help of an isotonic simple straw lever (magnification × 10).

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For quantitative tocolytic studies, cumulative concentration response curves of β₂ adrenoceptor agonists, namely albuterol (salbutamol) and terbutaline were constructed on prostaglandin F₂α (0.8 μM), oxytocin (0.004 IU/ml), potassium chloride (30 mM) and barium chloride (200 μM) pre-contracted perimetrial strips. A minimum time period of 1.0 to 1.5 hr was allowed to elapse between the recording of two successive concentration response-curves and the perfusion fluid was changed every 10-15 min.

The responses to each concentration of the agonist against different spasmsogens were plotted as per cent of the maximum relaxation response versus molar concentration of the drug. The median effective concentration (EC₅₀) of each agonist against different spasmsogens(s) was calculated from the cumulative-concentration response curves and the 95 per cent confidence limits of EC₅₀ were determined. The data were subjected to statistical analysis to determine the significant differences.

Results

Effect of albuterol and terbutaline on PGF₂α precontracted uterine tissues—The isolated uterine strips of dioestrous and pregnant buffaloes pre-contracted with PGF₂α (0.8 μM) were effectively relaxed in a concentration-dependent manner by albuterol and terbutaline. The minimum threshold concentration (MTC), the maximum concentrations required to induce maximal relaxation along with the cumulative dose response curves (CDRCs) of albuterol and terbutaline are shown in Fig. 1A. The EC₅₀ values along with confidence limits of albuterol and terbutaline against PGF₂α are presented in Table 1.

Effect of albuterol and terbutaline on oxytocin precontracted uterine tissues—Both albuterol a terbutaline relaxed oxytocin (0.004 IU/ml) precontracted isolated dioestrous and pregnant buffalo myometrium in a concentration-dependent manner. The CDRCs showing the MTC and the concentration of albuterol or terbutaline required to induce maximum relaxation are shown in Fig. 1B. The EC₅₀ values with 95 per cent confidence limits of albuterol and terbutaline are summarized in Table 1.

Effect of albuterol and terbutaline on potassium chloride-precontracted uterine tissues—Albuterol and terbutaline relaxed the potassium chloride (30 mM) pre-contracted uterine tissues from both dioestrous and pregnant buffaloes in a concentration-dependent manner. The MTC and the maximal concentrations required to induce maximal relaxation along with the CDRCs of albuterol and terbutaline are shown in Fig. 2A. The EC₅₀ values with 95 per cent confidence limits of these agonists are presented Table 1.

Effect of albuterol and terbutaline on barium chloride precontracted uterine tissues—Barium chloride (200 μM) pre-contracted uterine tissues from both dioestrous and pregnant buffaloes were effectively relaxed in a concentration-dependent manner by albuterol and terbutaline. The CDRCs of these agonists showing the MTC and the concentrations required

<table>
<thead>
<tr>
<th>Spasmoden (conc in bath fluid/ml)</th>
<th>EC₅₀ values of albuterol (M)</th>
<th>EC₅₀ values of terbutaline (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant Dioestrous</td>
<td>Pregnant Dioestrous</td>
</tr>
<tr>
<td>PGF₂α (0.8 μM)</td>
<td>(4.80 x 10⁻¹³ - 7.92 x 10⁻¹³)</td>
<td>(3.29 x 10⁻¹⁴ - 4.76 x 10⁻¹⁴)</td>
</tr>
<tr>
<td>Oxytocin (0.004 IU/ml)</td>
<td>2.51 x 10⁻¹³</td>
<td>1.78 x 10⁻¹²</td>
</tr>
<tr>
<td>KCl (30 mM)</td>
<td>(2.15 x 10⁻¹⁰ - 2.94 x 10⁻¹⁰)</td>
<td>(1.32 x 10⁻¹³ - 2.29 x 10⁻¹³)</td>
</tr>
<tr>
<td>BaCl₂ (200 μM)</td>
<td>3.92 x 10⁻¹²</td>
<td>1.77 x 10⁻¹²</td>
</tr>
<tr>
<td></td>
<td>(3.39 x 10⁻¹² - 4.55 x 10⁻¹²)</td>
<td>(9.36 x 10⁻¹³ - 2.0 x 10⁻¹²)</td>
</tr>
</tbody>
</table>

Data in parentheses indicate the confidence limits of EC₅₀ values
Means in a row with different capital superscripts indicate significant difference between the stages of uterus
Means in a column with different small superscripts indicate significant difference between the spasmsogens
induce maximal relaxation are shown in Fig. 2B. The EC₅₀ values with 95 per cent confidence limits of albuterol and terbutaline are presented in Table 1.

**Comparative assessment of the tocolytic efficacy of albuterol and terbutaline**

**Albuterol**—The overall comparative assessment of the EC₅₀ values of albuterol (Table 1) on the isolated uterine strips from the dioestrous and pregnant buffaloes, pre-contracted with different spasmogens, revealed that irrespective of the state of uterus, whether gravid or non-gravid, KCl-depolarized tissues required the highest concentration of albuterol for relaxing the maximally contracted tissues by 50 per cent. Further, compared to the uteri of pregnant buffaloes, the uteri of dioestrous stage animals were more sensitive to the relaxant effect of albuterol irrespective of the spasmogens(s) used except in case of OT-precontracted tissues where the sensitivity of uteri of both the stages did not differ significantly.

**Terbutaline**—Comparative assessment of EC₅₀ values of terbutaline (Table 1) on the dioestrous and

![Fig. 1 - Cumulative concentration response-curves of albuterol and terbutaline against (A) PGF₂α (0.8 μM) and (B) oxytocin (0.004 IU/ml) pre-contracted isolated uterine strips of dioestrous and pregnant buffaloes. [Vertical bars denote the SE of mean values (n=4-6)]]
pregnant buffaloes uterine strips pre-contracted with
different spasmo gens revealed that irrespective of the
stage of uterus, whether gravid or non-gravid, BaCl₂-
depolarized tissues required comparatively lowest
concentration of terbutaline and there was no signifi­
cant difference in the sensitivity of uteri of both the
stages depolarized with BaCl₂ to terbutaline-induced
relaxation. The uteri of pregnant buffaloes, compare
to those of dioestrus animals, pre-contracted w i
oxytocin and KCl were significantly less sensitive
terbutaline-induced tocolysis. On the contrary, uterir
strips of pregnant animals pre-contracted with PGF
were significantly more sensitive to the relaxant effe
of terbutaline.

Fig. 2—Cumulative concentration response-curves of albuterol and terbutaline against: (A) KCl (50 mM) and (B) BaCl₂ (200 μM)
pre-contracted isolated uterine strips of dioestrus and pregnant buffaloes. [Vertical bars denote the SE of mean values (n=5-9)]
Rank order potency of albuterol and terbutaline against different spasmogens—Based on the comparative EC_{50} values of albuterol and terbutaline on the uterine strips from the pregnant buffaloes, the rank order potency of albuterol was OT > BaCl_{2} > PGF_{2α} > KCl while that of terbutaline was BaCl_{2} > PGF_{2α} > KCl > OT. Pharmacological response of uterine strips of the dioestrous stage animals was different from that of the pregnant animals as the rank order potency of albuterol on dioestrous stage uterus was PGF_{2α} > BaCl_{2} > OT > KCl and that of terbutaline was BaCl_{2} > OT > KCl > PGF_{2α}. Uterine strips of pregnant animals pre-contracted with oxytocin were most sensitive to albuterol and least to terbutaline, while dioestrous stage animals uteri pre-contracted with PGF_{2α} were most sensitive to albuterol and almost least to terbutaline.

Discussion

Studies on the isolated uterine strips from both the dioestrous and pregnant buffaloes revealed that PGF_{2α}, OT, KCl or BaCl_{2} pre-contracted tissues were effectively relaxed in a concentration-dependent manner by albuterol and terbutaline, thus, convincingly suggesting that both albuterol and terbutaline are promising tocolytic for buffaloes as has been reported in rats and mares. Therapeutic tocolytic efficacy of certain β_{2} adrenoceptor agonists has already been proved in certain species of animals in veterinary medicine.

Critical evaluation of the rank order potency of albuterol against different spasmogens, both on the dioestrous and pregnant animal uterine strips revealed that irrespective of the state of uterus, KCl-depolarized tissues required the highest concentrations of albuterol to induce uterine relaxation. However, the rank order potency of terbutaline was slightly different from that of albuterol as in the rank order, KCl-depolarized tissues were at third position instead of fourth.

Overall comparison of the tocolytic efficacy of albuterol and terbutaline suggested that dioestrous uterine strips were equi-responsive or slightly to significantly more sensitive irrespective of the spasmogens used except that of PGF_{2α}, wherein uterine strips from pregnant buffaloes were more sensitive to the relaxant effect of terbutaline. Further, the uterine strips of pregnant animals pre-contracted with OT were more responsive to albuterol, while BaCl_{2} or PGF_{2α} precontracted tissues were sensitive to terbutaline. The observations on the differences in the sensitivity of uteri of pregnant and dioestrous animals to the tocolytic efficacy of these β_{2} adrenoceptor agonists indicate that in addition to the selective β_{2} adrenergic receptor, involvement some other subtle cellular or molecular mechanisms may be influencing the tocolytic efficacy in buffaloes.

Beta adrenoceptor agonists relax smooth muscles by increasing the intracellular levels of cyclic AMP and/or increasing K^{+} conductance. The latter process causes hyperpolarization thus, resulting in inhibition of voltage-operated calcium channels and decrease in calmodulin-activation. Isoproterenol-induced relaxation of rat myometrium is due to its hyperpolarizing effect which is associated with an increase in K^{+} conductance. Isoproterenol-induced myometrial relaxation in rats has been reported to be mediated via outward K^{+} current through Ca^{2+}-activated K^{+} channels and thus suggested that isoproterenol-induced tocolytic effect may also be due to outward K^{+} current and hyperpolarization of myometrium.

Potassium chloride (30mM)-depolarized uterine preparations required comparatively higher concentrations of albuterol and terbutaline to induce relaxation. Presence of high concentrations of K^{+} in the biophase and/or the perfusion fluid may have interfered with the albuterol or terbutaline-induced outward K^{+} current and thus the higher concentrations of these selective β_{2} adrenoceptors might have been required for relaxing the KCl-precontracted uterine tissues. Thus, the EC_{50} values of albuterol and terbutaline against KCl-depolarized tissues were significantly higher compared to those against other spasmogens. Ishine et al. have also observed that high K^{+} concentration in the medium significantly inhibit isoprote­senol-induced relaxation. Based on the similarity of our observations in the present study with the data available in the literature, indirectly the possibility of existence of K_{Ca} channels in buffalo myometrium and their activation by β_{2} adrenergic receptor agonists can not be ruled out. Anwer et al. have also reported the existence of prominent K_{Ca} channels in the pregnant rat myometrium which are regulated by β_{2} adrenergic receptor stimulation. However, to establish the existence and investigate the properties of K_{Ca} channels in the uterus of buffaloes, further detailed studies using whole cell current recording or specific K_{Ca} channels antagonists are required.

References


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