The Effect of Spironolactone of the Rat Uterus

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Isolated rat uterus preparations were used in this study. Ritodrine hydrochloride (Rit), isoxsuprine (Isox), and spironolactone (SP) were applied on the uterus which has spontaneous activity. In similar doses they produced various inhibitory effects. Also, when oxytocin (Oxt) or acetylcholine (Ach) was conducted to the isolated preparations, contractions with high tonus and low amplitude occurred. On this model we investigated the inhibitory effect of spironolactone on the contractions. We conclude that spironolactone can be utilized on the uterus as a uterine contraction inhibitory agent in well regulated doses.

(Key Words: Spironolactone, rat uterine horn)

INTRODUCTION

Preterm delivery is an important causative factor perinatal mortality. The etiology of preterm labor is not clear although there is evidence that genital infection is an important factor (1). Through the years, many treatment regimens have been employed to arrest labor. Commonly used agents are magnesium sulphate, beta adrenergic receptor stimulants (Isox, Rit, terbutaline), and antiprostaglandins (8, 9).

SP is an aldosterone antagonist that is used in the treatment of congestive heart failure, hepatic ascites, primary aldosteronism, essential hypertension, ascites, familial male precocious puberty, premenstrual tension, hirsutism and acne. It is extensively used in the clinic as a potassium-sparing diuretic and as an antihypertensive agent. SP has a positive inotropic effect and this effect is related to several ion conductances especially a decrease in potassium conductance (2, 6, 7, 10, 13, 14, 17, 18).

We were curious about the other possible effects of SP on the human being. Therefore, we planned the present study to investigate the effect of SP on uterine contractions. While we were testing the effect of SP on the rat uterine horn, for comparison, we also investigated the response of the muscles of the ileum and the trachea in SP.

MATERIALS AND METHODS

This study was performed at the Erciyes University Experimental and Clinic Research Center, Kayseri, Turkey. The animals used in the study were female 30 Swiss-Albino rats and each weighed 180-240 g. Twenty four hours before beginning the study to the each rat 5 mg/kg diethylstilbestrol was injected intraperitoneally into each ratin order to stimulate an estrus period. The rats were killed by decapitation. The abdomens of the rats were opened through a ventral midline incision, and uterine horns were identified and exposed. Each horn was excised and used for isolated uterine preparation. Ringer-Locke solution was used. Ten ml of isolated organ unit temperature was kept 37°C during the procedures and it was aerated by carbogen (95% O₂ plus 5% CO₂) continuously. One end of the horn was applied to the transducer and the other end was connected to the bottom of the isolated organ bath unit. Control and experimental motility traces were recorded by TB 611 T Force Displacement Transducer using 6000 Nihon Kohden Polygraphic System. Similarly, the ileum was exposed and 2.5-3 cm strip was excised without the mesentary, and used for isolated ileum preparation as in uterine horn.

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Also, after the cervical incision the trachea was excised and approximately 2 cm of trachea was obtained. The trachea segments were not separated, and used for isolated trachea preparations as in uterine and ileum preparations as described previously [16].

After recording spontaneous uterine activity the dose related effects of Rit (Prepar, 5 mg/5ml (1.7 × 10⁻⁶ M), Eczacinasi Co., Istanbul, Turkey), Isox (Duvasilman, 10 mg/2 ml (3.2 × 10⁻⁵ M), Eczacinasi Co., Istanbul, Turkey) and SP (Potassium Canrenoate, powder, Searles-Sanofi Chimie Co., France) on the uterus contractions were explored. The chemical structure of Rit is C₁₇H₂₉N0₃, the structure of Isox is C₁₈H₂₉N₂O₃, and the structure of Potassium Canrenoate is K-C₂₂H₂₉O₃. For each preparation the threshold level of the agent was obtained. Then, over the threshold level dose the agent was applied.

After setting of the isolated organ bath unit we waited for 60 minutes to obtain spontaneous activity stabilization. Agents were added to the solution in cumulated doses and these were passed to the myometrium by diffusion. SP and the well known tocolytic agents Rit and Isox were applied to the different preparations to compare the responses. To control and provoke the isolated preparations we added Oxt (Synpitan, 51U/ml, Deva Co., Istanbul, Turkey) and Ach (Acetylcholine Bromid, 10 mg/2 ml (2.2 × 10⁻² M), Ciba-Geigy Co., Istanbul, Turkey). The preparations increased their contractions in response to Oxt and Ach.

After each application of inhibitory agents, preparations were washed at least three times and the response traces were recorded during 20 minute intervals. The traces obtained from the control and the experimental groups were compared according to their amplitude, frequency and inhibition percentage.

Statistical analyses were performed with the paired Student t-test.

RESULTS

The statistical comparison of SP, Rit, Isox, and inhibitory effects on the isolated rat uterine horn preparations are presented in Table I. The responses for the applied doses of the agents on the uterine horn preparations were different. 3.4 × 10⁻⁶ M of Rit dose created 100% inhibition, 2.4 × 10⁻⁵ M of Isox dose created 68% inhibition, and 2.4 × 10⁻⁶ M of SP dose created 16% inhibition. However, the frequencies were not too much effected except for total inhibition.

Table II and Fig. 1 show inhibitory effects of SP on the uterine horn, the ileum, and the trachea preparations at different doses. 6.0 × 10⁻⁶ M dose of SP created 100% inhibition on the amplitude of the ileum, 80% inhibition on the uterine horn, and 20% inhibition on the trachea. 7.2 × 10⁻⁶ M dose of SP created total inhibition on the uterine horn and the ileum, but only 25% inhibition was obtained on the trachea. 2.4 × 10⁻⁵ M dose of SP created total inhibition on all of the preparations.

The inhibition traces of Rit, Isox, and SP on the rat uterine horn contractions are shown in Figures 2-4. All of the agents inhibited the uterine horn motility in different doses. Inhibitory effect on the amplitudes were clearly observed, but this effect on the frequencies were not too much.

Table I The effect of SP, Rit, Isox on the isolated rat uterus motility

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (mg)</th>
<th>Mole concentration</th>
<th>Amplitude (mm)</th>
<th>Frequency (%)</th>
<th>Amplitude Inhibition % p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP</td>
<td>10</td>
<td>4</td>
<td>1.2 × 10⁻⁶</td>
<td>25.0</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8</td>
<td>2.4 × 10⁻⁶</td>
<td>25.0</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>1.7 × 10⁻⁶</td>
<td>8.5</td>
<td>0.60</td>
</tr>
<tr>
<td>Rit</td>
<td>10</td>
<td>10</td>
<td>3.4 × 10⁻⁶</td>
<td>10.0</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>1.6 × 10⁻⁵</td>
<td>13.0</td>
<td>0.85</td>
</tr>
<tr>
<td>Isox</td>
<td>10</td>
<td>7.5</td>
<td>2.4 × 10⁻⁵</td>
<td>12.0</td>
<td>0.88</td>
</tr>
</tbody>
</table>

NS: Statistically not significant
TI: Total inhibition
The uterine horn motilities, which were provoked by Oxt and Ach were totally inhibited by cumulated doses of SP (Fig. 5, 6). The uterine horn motility which was provoked by Oxt was inhibited totally with $9.0 \times 10^{-6}$ M SP dose, and which was provoked by Ach was inhibited totally with $1.2 \times 10^{-5}$ MSP dose.

**DISCUSSION**

At the present time preterm labor and delivery are still an unsolved health problem. Aims to find an agent which can arrest the labour with the least side effect are continuing. In our study we used SP on the rat uterine horn and obtained an inhibitory effect on its spontaneous and stimulated activity.

The uterus is a visceral smooth muscle and it has stable motility and spontaneous activity. The results of the application of SP, Rit, and Isox, on the spontaneous activity of the uterus

<table>
<thead>
<tr>
<th>Table 2</th>
<th>The effects of SP on the uterine horn, the ileum, and the trachea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uterine horn (n:10)</td>
</tr>
<tr>
<td></td>
<td>Amplitude Frequency Amplitude Frequency Amplitude Frequency</td>
</tr>
<tr>
<td></td>
<td>(mm)                (sec)</td>
</tr>
<tr>
<td>Control</td>
<td>25                   0.7</td>
</tr>
<tr>
<td>SP</td>
<td>7.2 $\times 10^{-6}$ M</td>
</tr>
<tr>
<td>Effective</td>
<td>6.0 $\times 10^{-6}$ M</td>
</tr>
<tr>
<td>doses</td>
<td>2.4 $\times 10^{-5}$ M</td>
</tr>
</tbody>
</table>

TI: Total inhibition  
NE: Non effective

![Fig. 1](image1.png)  
**Fig. 1** Shows the inhibitory effects of SP on the ileum, trachea, and uterus smooth muscles.

![Fig. 2](image2.png)  
**Fig. 2** Shows the inhibitory effect of Rit on the spontaneous activity of the uterus.
the ileum and the trachea were shown in Figure 1-4. The statistical comparison is shown in Table I. At the effective dose of SP, inhibition levels were shown in Table II. By provoking spontaneous activity of the uterine horn by Oxt and Ach (Fig. 5, 6) which are intrinsic agents, we showed that SP has inhibitory effect on the increased tension and frequency of the uterine horn motility. We could not discuss this result, because there is no report on this subject in the literature.

A steroid lactone SP is a competitive aldosterone antagonist. SP activity in the cell is related to its effect on the energy metabolism by inhibiting ATP formation. Also, it inhibits the connection of aldosterone to the specific mineralcorticoid receptor. SP reverses the electrolyte regulation effect of aldosterone. The inhibition in the cellular activity is dependent on the decreased potassium concentration. In addition, SP reduces magnesium extraction and inhibits the activity of Sodium-Potassium-ATPase. Therefore, the energy for the resting membrane potential is obtained (3, 4, 5, 11, 12, 15, 19, 20). The inhibitory effect of SP on the uterine horn motility was mainly on the amplitude, and this effect was not too much on the frequency. We think that this different effect is related to mechanism of inhibition, which is probably created by actin-myosin and not by a neurogenic pathway.

In conclusion, our experimental study
showed that in a dose related manner SP has a good inhibitory effect on the rat uterine horn and also on the smooth muscle oragans; ileum and trachea. Further studies are required to establish its efficacy and safety in arresting preterm labour in the human.

REFERENCES