Pharmacological Investigation of Ritodrine Hydrochloride, a Beta₂-Adrenoceptor Stimulant

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Accepted August 24, 1984

Abstract—Ritodrine hydrochloride (ritodrine) has been effectively prescribed for the prevention of premature labor. The present study was carried out to investigate the mode of action of ritodrine on the uterus and heart in comparison with those of isoxsuprine and isoproterenol. 1) Ritodrine (10⁻⁸–10⁻⁶ M) suppressed the spontaneous motility of pregnant rat uterus and showed positive chronotropic action at the doses of 10⁻⁶–10⁻⁴ M in guinea-pig atria. 2) In the Ca²⁺-free, K⁺-rich Tyrode solution, ritodrine suppressed the Ca²⁺ induced contracture of pregnant rat uterus, while it potentiated the carbachol induced contraction. 3) Ritodrine increased the amount of cyclic AMP in the uterus but not in heart. This action of ritodrine was suppressed by pretreatment with propranolol (10⁻⁶ M). 4) These results suggest that ritodrine causes actions through activation of cyclic AMP production, as in the case of isoproterenol, and it acts more selectively on β₂-adrenoceptors than on β₁-adrenoceptors.

A significant proportion of perinatal mortality is attributed to low birth weight infants because of preterm labor (1). The agents which inhibit preterm labor are mainly the tocolytics which suppress abnormal uterine contractions (2). Recently, adrenergic β-stimulants are used for inhibition of preterm labor (3, 4).

Two types of β-receptors have been described. Beta₁-receptors are distributed predominantly in the heart, small intestine and adipose tissue, whereas β₂-receptors are found in the smooth muscle of the uterus, blood vessels and bronchioles (5).

Ritodrine hydrochloride (ritodrine) is reported to produce its effects on the uterus through activation of β₂-receptors (6, 7). Because many β-stimulants, including ritodrine, have both β₁ and β₂-activity, the severity of β₁-action is very important in causing the side effect of tachycardia (8). It is necessary to investigate the pharmacological properties of ritodrine such as β₁- and β₂-stimulating actions. Furthermore, it is very important to analyze the tocolytic mechanism of ritodrine.

Izumi and Kishikawa (9) showed that ritodrine suppressed pregnant rat myometrium and induced hyperpolarization of the membrane potential. Recently, it has been reported that the relaxation of smooth muscle caused by β-stimulants is correlated with intracellular Ca²⁺ changes through cyclic AMP systems (10, 11).

In this experiment, we intended to investigate the pharmacological properties of ritodrine, in comparison with isoxsuprine hydrochloride (isoxsuprine) and isoproterenol hydrochloride (isoproterenol).

Materials and Methods
Pregnant Wistar strain rats and Hartley strain guinea-pigs were used. In rats, the zero day of pregnancy was estimated by the presence of a vaginal plug after cohabitation overnight with male rats. The experiments were carried out on preparations excised from the rats on the 19th day of gestation.

1. Inhibitory action on the uterus and positive chronotropic action: Preparations of the uterus were dissected into pieces that were 2–3 mm wide and 18–20 mm long,
excluding the placental region, cervical region and vicinity of ovaries. These preparations were suspended in a 20 ml organ bath containing Krebs-Henselte bicarbonate solution at 37°C, and the solution was bubbled with a mixture of 95% O₂ and 5% CO₂. Initially, the preparation was loaded with 0.5 g. Contractions of the preparation were recorded isometrically on an ink-writing rectigraph (Type 8S, Sanei) through a force-displacement transducer (SB-1T, Nihon Kohden).

Isolated right artia from guinea-pigs were suspended in a 20 ml organ bath containing Krebs-Henselte bicarbonate solution at 37°C, bubbled with a mixture of 95% O₂ and 5% CO₂. Initially, the preparation was loaded with 0.5 g. Spontaneous beats were led out through a force-displacement transducer, and the number of beats were read from a cardiotachometer (2140, Sanei), triggered by contractions of the artia. The pD₂ values were calculated by the previously described method (12).

2. Effects on cyclic AMP contents of the uterus and heart in pregnant rats: Preparations of the uterus in pregnant rats were dissected longitudinally into pieces that were 4-5 mm long and 3-4 mm wide. After weighing the tissue, the preparation was incubated in the bubbled Tyrode solution for 50 min at 37°C. Then, it was kept in a glass homogenizer containing 0.9 ml of Tyrode solution with 10 mM of theophylline added at 37°C. After incubation for 5 min, the drugs to be tested were applied and incubated for 5 min. The reaction in the homogenizer was stopped by boiling for 10 min. The preparation was homogenized and centrifuged at 3,000 rpm for 15 min (KR-40, Kubota). The supernatant (0.5 ml) was used to measure the effect of drugs on the content of cyclic AMP. To observe the effects of β-adrenoceptor blockade, 10⁻⁶ M of propranolol was added to the test solution 90 sec before the application of theophylline (10⁻² M) and the drugs to be tested. The final volume was adjusted to 1 ml.

The left ventricular heart muscle was sliced in pieces that were 0.2-0.3 mm thick and about 30 mg in weight, excluding the apex, in cold Tyrode solution bubbled with a mixture of 95% O₂ and 5% CO₂. The procedure to measure the cyclic AMP was the same as that for the uterus described above. The contents of cyclic AMP were measured by the protein binding assay method as described previously (13). The contents of cyclic AMP were expressed as pmol/100 mg of wet tissue weight. The pD₂ values were calculated by the previously described method (12).

3. Effects on contractions induced by carbachol and calcium: The experiments were carried out using uterine muscle strips from rats on the 19th day of gestation. The tissues were dissected into pieces that were 13-15 mm long and 3-4 mm wide, excluding the placental region and vicinity of the ovaries.

These preparations were kept in an organ bath containing Tyrode solution bubbled with air at 37°C, and the nutrient solution was changed every 20 min for 2 hr. Then, the nutrient solution was changed to high K⁺ and Ca²⁺-free Tyrode solution (pH 7.4) at 20°C according to Ohashi’s method (14).

When the response was induced by carbachol, it was added to the organ bath at a final concentration of 1 mM. After application of carbachol, the preparation was washed by high K⁺ and Ca²⁺-free Tyrode solution at least 3 times for 5 min, and the next response was evoked 10 min later. When the response was induced by calcium, CaCl₂ was added to the organ bath at a final concentration of 0.5 mM. After the contractile response reached a plateau, the preparation was washed by high K⁺ and Ca²⁺-free Tyrode solution at least 3 times, and the next response was evoked 20 min later. Carbachol and calcium contractions were repeated 3 times, and drugs to be tested were added to the organ bath. Five min after the application of each drug, contraction was induced by CaCl₂. Ten min after washing of the preparation, carbachol was added to the organ bath.

Drugs used in this experiment and the compositions of the nutrient solutions were as follows: drugs used were ritodrine HCl (Duphar), isoxsuprine HCl (Duvadilan, Daichi), isoproterenol HCl (Nakarai), carbachol (Aldrich), verapamil (Knoll), propranolol (Inderal, ICI-Sumitomo) and cyclic
AMP assay kit (Amersham). High K⁺ and Ca²⁺-free Tyrode solution (mM) contained KCl, 139.7; MgCl₂, 1.0; Glucose, 5.0 and Tris-maleate buffer, 5. Tyrode solution (mM) contained NaCl, 137; KCl, 2.7; MgCl₂, 1.0; CaCl₂, 1.8; NaHCO₃, 20; NaH₂PO₄, 1.0 and Glucose, 5.5. Krebs-Henselite bicarbonate solution (g/l) contained NaCl, 34.5; KCl, 1.75; CaCl₂, 2.75; KH₂PO₄, 0.8; MgSO₄·7H₂O, 1.45; Glucose, 5 and NaHCO₃, 10.5.

Results

Inhibitory action on the uterus and positive chronotropic action: Effects of the drugs tested are shown in Fig. 1, represented as a dose-response relationship according to the contractile amplitude. Ritodrine, isoxsuprine and isoproterenol began to show inhibitory action on uterine motility at concentrations of 3×10⁻⁸ M, 3×10⁻⁸ M and 3×10⁻¹¹ M, respectively. Maximal suppression was seen with 10⁻⁶ M of ritodrine, 3×10⁻⁶ M of isoxsuprine and 10⁻⁸ M of isoproterenol. The pD₂ values calculated from dose-response relationships are presented in Table 1. The effects of these drugs on guinea-pig right atria are shown in Fig. 2 as the dose-response relationship with respect to chronotropism. Isoproterenol showed the strongest positive chronotropic action, and the effect of ritodrine was almost the same as that of isoxsuprine. The pD₂ values calculated from dose-response relationships are shown in Table 1. In Table 1, selectivity is shown as the difference between pD₂ values in the uterus and atria. The order of selectivity in the uterus was as follows: ritodrine > isoproterenol > isoxsuprine.

Effects on cyclic AMP content: Effects of isoproterenol, ritodrine and isoxsuprine on

![Graph](image_url)

Fig. 1. Effects of ritodrine, isoxsuprine and isoproterenol on spontaneous motility in pregnant rat uterus (19–20th days of gestation), (N=12–14). Vertical bars indicate S.E.M.

| Drug         | Pregnant rat uterus (19–20th) | Guinea-pig atria (n=6) | Selectivity |
|--------------|-------------------------------|------------------------|-------------|
|              | Spontaneous motility (n=12–14) | (A) Amplitude (F) Frequency | (C) Chronotropism | (A)-(C) | (F)-(C) |
| Ritodrine    | 7.24±0.05 (7.40±0.15)         | 5.70±0.11              | 1.54        | 1.70    |
| Isoxsuprine  | 6.52±0.17 (6.99±0.25)         | 5.84±0.02              | 0.68        | 1.15    |
| Isoproterenol| 9.79±0.23 (9.97±0.17)         | 8.60±0.11              | 1.19        | 1.37    |

Beta₂-selectivity was calculated as (A)-(C) and (F)-(C).
cyclic AMP content in pregnant rat uterus are indicated in Fig. 3. Isoproterenol began to increase the content of cyclic AMP at a concentration of $10^{-8}$ M and increased it dose-dependently at higher concentrations. A concentration of $10^{-6}$ M of isoproterenol showed the maximal response, and the concentration of cyclic AMP was about 3.8 times higher than that in the control. This response was significantly different from that of the control ($P<0.001$). Ritodrine began to show increases of cyclic AMP content at $10^{-5}$ M and reached a plateau at $10^{-3}$ M. At $10^{-4}$ M, ritodrine showed an increase of cyclic AMP to 2.6 times than that of the control. These responses to ritodrine above $10^{-5}$ M were significantly different from the control level at $P<0.001$. Isoxsuprine showed minimal response and $10^{-4}$ M only increased cyclic AMP to 1.9 times that of the control. This response to $10^{-4}$ M isoxsuprine was significantly different from the control level at $P<0.01$. The $pD_2$ values calculated from these dose-response relationships were $6.96 \pm 0.16$ for isoproterenol, $5.17 \pm 0.20$ for ritodrine, and $4.89 \pm 0.27$ for isoxsuprine. The effects of the three drugs tested on cyclic AMP content were suppressed by pretreatment with $10^{-6}$ M propranolol (Fig. 3).

Figure 4 shows the effects of isoproterenol, ritodrine and isoxsuprine on cyclic AMP production in the pregnant rat heart. Isoproterenol significantly increased cyclic AMP content at all concentrations used. The
amount of cyclic AMP after treatment with $10^{-6}$ M isoproterenol was 2.5 times that of the control, but it decreased with $10^{-5}$ M isoproterenol. Ritodrine showed the maximal production of cyclic AMP at $10^{-5}$ M, but the concentration was only 1.4 times that of the control. Isoxsuprine also increased the cyclic AMP content dose-dependently, and it was 1.5 times that of the control with $10^{-6}$ M isoxsuprine.

As shown in Table 2, the pD$_2$ values of isoproterenol in the uterus and heart were 6.96±0.16 and 7.05±0.19. The pD$_2$ value of ritodrine in the uterus was 5.17±0.20, but it could not be calculated in the heart because ritodrine did not show dose-response relationships. Isoxsuprine showed pD$_2$ values of 4.89±0.27 and 6.44±0.33 in the uterus and heart.

Analysis of uterus muscle relaxant action:
The uterus preparation kept in Tyrode solution at 37°C showed transient contracture when the nutrient solution was changed to high K$^+$ and Ca$^{2+}$-free Tyrode solution at 20°C and returned to the resting tone level within 20 min. One mM carbachol induced transient contraction in this preparation.

After washing with high K$^+$ and Ca$^{2+}$-free Tyrode solution, no more contractions were seen (Ca$^{2+}$-depleted preparation). These responses are shown in Fig. 5A. The Ca$^{2+}$ depleted preparation showed prolonged contracture by 0.5 mM of CaCl$_2$ (Ca$^{2+}$-treated preparation). These contractures reached a plateau within 10 min. After washing with high K$^+$ and Ca$^{2+}$-free solution, transient contraction was seen by the application of carbachol (Fig. 5A). Therefore, carbachol and CaCl$_2$ induced contractile responses were evoked alternately. These responses were repeated constantly about 5 to 6 times by CaCl$_2$ and carbachol.

As shown in Fig. 5B, verapamil dose-dependently suppressed both Ca$^{2+}$ and carbachol induced contractile responses. These suppressive effects of verapamil were prolonged and little recovery was seen after treatment with high concentrations. Treatment with $10^{-7}$ M ritodrine suppressed Ca$^{2+}$ induced contracture, while it increased carbachol induced contraction (Fig. 5C). Treatment with $10^{-9}$ M isoproterenol similarly suppressed CaCl$_2$ induced contracture and enhanced carbachol induced contraction (Fig. 5D). Both responses were little affected by $10^{-8}$ M–$10^{-4}$ M of isoxsuprine. Figure 6 shows the dose-response relationships of ritodrine and isoproterenol on Ca$^{2+}$ contracture and K$^+$ contraction. These effects of ritodrine and isoproterenol on Ca$^{2+}$ and carbachol induced contractile responses were almost eliminated by pretreatment with $10^{-6}$ M of propranolol.

Discussion
Lands et al. (5) reported that adrenergic

| Table 2. The pD$_2$ values of ritodrine, isoxsuprine and isoproterenol for cyclic AMP content in pregnant rat uterus and heart (19th day of gestation) |
|-----------------|-----------------|
|                 | Uterus (n=8)    | Myocardium (n=8–9) |
| Ritodrine       | 5.17±0.20       | –                 |
| Isoxsuprine     | 4.89±0.27       | 6.44±0.33         |
| Isoproterenol   | 6.96±0.16       | 7.05±0.19         |

*: Unable to calculate the pD$_2$ value.
β-receptors were pharmacologically characterized as the β1-type and β2-type and that β1-receptors were mainly distributed in the heart and β2-receptors in the bronchioles and uterus. Some β-stimulants used in obstetrics to prevent premature labor acted on both the uterus (β2-receptors) and heart (β1-receptors). These drugs caused tachycardia as a side-effect (8). Therefore, selectivity of β2-action is very important for tocolytic agents.

To investigate this selectivity, it is useful to compare the pharmacological potency of drugs using specific organs such as the heart (β1), trachea (β2) and uterus (β2) (15). It is also useful to compare the production of cyclic AMP in specific organs such as the heart, trachea and uterus (16).

In this experiment, ritodrine, isoproterenol and isoxsuprine showed more potent uterine muscle relaxant actions than positive chronotropic actions in the atria. The actions of ritodrine, isoproterenol and isoxsuprine in the uterus were 30–50, 20 and 6–10 fold those in the atria, respectively. The fact that ritodrine selectively showed 30–50-fold higher action in the uterus than in the atria suggested that it has wide safety margin.
when used to prevent premature labor in obstetrics. The concentrations of isoproterenol which increased cyclic AMP production were almost the same in both the heart and uterus. Ritodrine clearly increased cyclic AMP content in uterine muscle, but it had little effect on cyclic AMP content in the heart muscle. Isoxsuprine showed little effect on cyclic AMP contents in both the uterus and heart.

These results also suggested that ritodrine has selective effects on the uterus. Izumi and Kishikawa (9) reported that ritodrine suppressed uterine contractions and showed an increase in cyclic AMP production in pregnant rat uterus. They also reported that these actions of ritodrine were about 100 times less than those of isoproterenol, which was in close agreement with our results. However, there was a discrepancy between muscular relaxation and increase in cyclic AMP production by application of ritodrine and isoproterenol.

The minimum concentration of these drugs required to produce muscular relaxation was lower than that required to induce an increase in the amount of cyclic AMP. It appears that β-adrenoceptor stimulants do not act only through the cyclic AMP system. Ohashi et al. (14) reported that isoproterenol induced cyclic AMP production and relaxed smooth muscle by the enhancement of Ca²⁺ intake into intracellular storage sites. In this experiment, we confirmed that ritodrine and isoproterenol suppressed Ca²⁺ induced contraction, while it potentiated carbachol induced contraction in Ohashi's preparation. These responses were inhibited by pretreatment with propranolol. These results suggested that ritodrine acted on the cyclic AMP system and induced muscular relaxation through the enhancement of Ca²⁺ intake into intracellular storage sites, similar to the action of isoproterenol. Scheid et al. (17) suggested that adrenergic β-stimulants induced cyclic AMP production, triggering the activation of the Na⁺/Ca²⁺ pump and enhanced Ca²⁺ extrusion from intracellular sites.

Since muscular relaxation induced by ritodrine may not be solely due to activation of cyclic AMP production, i.e., direct inhibitory action on the contractile protein mechanism as in the case of calmodulin (18), further studies are necessary to explain the pharmacological mechanism of ritodrine. Izumi and Kishikawa (9) also reported that ritodrine induced hyperpolarization of membrane potentials and suggested that these effects might cause relaxation of smooth muscle.

In this experiment, we confirmed that ritodrine induced cyclic AMP production, suggesting the enhancement of Ca²⁺ intake into intracellular storage sites through activation of β-adrenoceptors and the more selective β₂-action than β₁-action of ritodrine.

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