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Pharmacological Characterization of KUR-1246, a Selective Uterine Relaxant

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ABSTRACT

The aim of the present study was to evaluate the efficacy and β2-adrenoceptor (AR) selectivity of KUR-1246, a new uterine relaxant. Inhibition of spontaneous or drug-induced uterine contractions by KUR-1246 was evaluated in pregnant rats and rabbits by an organ bath method or by a balloon method. The selectivity of KUR-1246 was assessed simultaneously in organs isolated from late-pregnant rats. The affinity of KUR-1246 for human β1-, β2-, and β3-ARs was determined using two radioligands. KUR-1246 suppressed both spontaneous and drug-induced contractions in isolated uteri, the rank order of potency being isoproterenol > KUR-1246 > terbutaline > ritodrine. ICI-118551 (selective β2-AR antagonist) competitively antagonized the KUR-1246-induced inhibition of spontaneous uterine contractions, but CGP-20712A (selective β1-AR antagonist) and SR-58894A (selective β3-AR antagonist) did not. All β-AR agonists tested produced significant inhibition of spontaneous uterine contractions in vivo: ED_{30} value for KUR-1246 was 0.13 μg/kg/min, a potency about 6 times and 400 times greater than that of terbutaline and ritodrine, respectively. In contrast, the positive chronotropic effect was minimal in KUR-1246-treated rats. KUR-1246 displaced radioligand binding to β1-, β2-, and β3-ARs, the pK_i values being 5.75 $\pm$ 0.03, 7.59 $\pm$ 0.08, and 4.75 $\pm$ 0.03 for β1-, β2-, and β3-ARs, respectively. For the selectivity of KUR-1246 for human β2-AR, we obtained values of 39.2 ([IC_{50} for β1-AR]/[IC_{50} for β2-AR]) and 198.2 ([IC_{50} for β3-AR]/[IC_{50} for β2-AR]), indicating an apparently higher affinity for human β2-AR than for other β-AR subtypes. The present study clearly demonstrated that KUR-1246 is a more selective β2-AR agonist than the drugs presently used for relaxing uterine muscle.

Preterm labor still remains one of the most serious problems in obstetric practice. For preventing or treating premature labor, β2-adrenoceptor (AR) stimulants, such as ritodrine and terbutaline, that inhibit contractions of uterine smooth muscle by stimulating the production of cytosolic cAMP via β2-AR, have been used extensively. However, the β2-AR agonists used at present are not particularly selective for uterine smooth muscle, and side effects on the cardiovascular and metabolic systems are frequent.

In recent years, the presence of β3-AR, an additional β-AR subtype, has been confirmed in various human tissues, including smooth muscle such as colon (Ponti et al., 1996), ureter (Park et al., 2000), and urinary bladder (Igawa et al., 1999). Moreover, a number of other investigators have reported that β1-AR preferentially exists in human and various rodent hearts (Juberg et al., 1985; Jones et al., 1989). Although it is not certain whether β1- or β3-AR is present and has functional roles in uterine smooth muscle cells, in human uterine smooth muscle β2-AR is the most abundant subtype in the later stages of gestation (Hayashida et al., 1982; Legrand et al., 1987).

In the present study, we characterized the pharmacological profile of a putative β-AR agonist, KUR-1246 (Fig. 1), paying special attention to its β2-AR selectivity over β3-AR, as well as over β1-AR. We evaluated both the efficacy and selectivity of this compound in the pregnant rat and rabbit. In addition, its β2-AR selectivity was confirmed in a receptor-binding assay using membrane preparations obtained from Sf9 cells expressing human β1- or β2-AR, and from SK-N-MC neuroblastoma cells expressing human β3-AR.

Materials and Methods

Animal and Animal Care

Albino rats of the Sprague-Dawley strain (SLC Co., Ltd., Hamamatsu, Japan) and rabbits of the New Zealand White strain (Kitayama Labes Co., Ltd., Ina, Japan) were used. They were housed in a constant-temperature room with a 12-h light/dark cycle. Virgin female rats were placed in separate cages with one male each and left overnight. Pregnancy was dated by taking the morning sperm-plug detection as day 0 of gestation. In the case of rabbits, the day of mating was taken as day 0 of gestation. The experiments in this study were conducted in accordance with institutional guidelines.

ABBREVIATIONS: AR, adrenoceptor; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

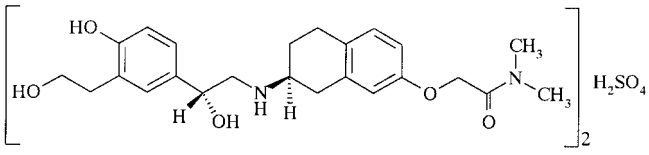

Fig. 1. Chemical structure of KUR-1246, (−)-bis(2-{[(2*S*)-2-({(2*R*)-2-hydroxy-2-[4-hydroxy-3-(2-hydroxyethyl)phenyl]ethyl}amino)-1,2,3,4-tetrahydronaphthalen-7-yl]oxy}-*N,N*-dimethyl-acetamide) monosulfate.

Drugs

The following drugs were obtained from commercial sources: ICI-118551 ((±)-1-[(2,3-dihydro-7-methyl-1*H*-inden-4-yl) oxy]-3-[(1-methylethyl) amino]-2-butanol hydrochloride) (Funakoshi, Tokyo, Japan), terbutaline hemisulfate and atenolol (Sigma, St. Louis, MO), (−)-isoproterenol hydrochloride (Nikken-Chemical, Tokyo, Japan), [^{3}H]CGP-12177 ((−)-[5,7-^{3}H]-[4-[3[(1,1-dimethylethyl) amino]-2-hydroxypropoxy]-1,3-dihydro-2*H*-benzimidazol-2-one]) and [^{125}I]iodocyanopindolol (PerkinElmer Life Science Products, Boston, MA), *dl*-propranolol hydrochloride (Nacalei tesque, Kyoto, Japan), oxytocin (Teikoku-zoki, Tokyo, Japan), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Pharmacia-Upjohn, Tokyo, Japan).

KUR-1246 (−)-bis(2-{[(2*S*)-2-({(2*R*)-2-hydroxy-2-[4-hydroxy-3-(2-hydroxyethyl)phenyl]ethyl} amino)-1,2,3,4-tetrahydronaphthalen-7-yl]oxy}-*N,N*-dimethyl-acetamide)monosulfate, ritodrine hydrochloride, (±)-bupranolol hydrochloride, and CGP-20712A (2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1*H*-imidazole-2-yl) phenoxy)-propyl)amino)ethoxy)benzamide monomethane sulfonate) were all synthesized in our laboratory.

Each drug was dissolved in buffer solutions (in vitro experiments) or physiological saline (in vivo experiments) just before the experiment.

In Vitro Functional Study

Effects of β-AR Agonists on Spontaneous or Drug-Induced Myometrial Contractions in Pregnant Rats and Rabbits. *Rats.* The experiments were carried out by the method of Kawarabayashi et al. (1996). Myometrial contractions were quantified as the sum of the amplitudes of all the contractions recorded over a 5-min period, and the percentage change (after versus before drug application) was calculated. Drug potency was expressed as the pD_2 value, which is the negative logarithm of the EC_{50} value (the 50% effective concentration of the β-AR agonist).

To assess the pharmacological profile of KUR-1246 on spontaneous myometrial contractions, effects of CGP-20712A (a selective β1-AR antagonist), ICI-118551 (a selective β2-AR antagonist), and SR58894A (a selective β3-AR antagonist) were examined. Each antagonist was added 15 min before treatment with KUR-1246. The results obtained were subjected to Schild plot analysis, and the pA2 values were obtained.

Rabbits. The experiments were performed using essentially the same method as that described above for the evaluation of spontaneous contractions in rats. Uterine tissues were obtained from rabbits on pregnancy day 29. Modified Krebs' solution (same as Krebs' solution contents except for KCl: 10.0 mM) and Krebs' solution were used in the experiments carried out to evaluate the effects on spontaneous myometrial contractions and oxytocin (10 mU/ml)-induced myometrial contractions, respectively. Myometrial contractions were quantified as the sum of the amplitudes of the contractions recorded over a 10-min period.

Effects of β-AR Agonists on Isolated Atria and Proximal Colon from Pregnant Rats. The chronotropic effects of KUR-1246 and other β-AR agonists were determined using atria isolated from pregnant rats on gestational day 21 by the method of Mattsson et al. (1982). Each drug was applied cumulatively to the bath solution, with the drug being added only when the chronotropic response had reached maximum at the previous concentration. Intrinsic activity was calculated as the ratio between the maximal increment in heart rate for a given drug and isoproterenol.

The inhibitory effects of the proximal colon isolated from pregnant rats on gestational day 21 by KUR-1246 and other β-AR agonists were examined according to the method of Bianchetti and Manara (1990), with minor modification. The colonic strips (10 mm long) were suspended in Tyrode's solution. Both 10^{-7} M CGP-20712A and 10^{-7} M ICI-118551 were present in the nutrient solution to block the β1- and β2-AR-mediated inhibitory effects of the agonists on the colon.

Receptor Binding Study

β-AR binding assays were carried out using membrane preparations obtained from Sf9 cells expressing human cloned β1- or β2-AR (PerkinElmer Life Science Products) or from SK-N-MC neuroblastoma cells expressing human nonrecombinant β3-AR (Receptor Biology, Inc., Manor Road, VA).

In the β1- and β2-AR binding experiments, the membrane preparation was suspended at protein concentration of 40 μg/ml in incubation buffer (75 mM Tris-HCl, 12.5 mM $MgCl_2$, and 2 mM EDTA; pH 7.4). In a test tube, 500 μl of incubation buffer containing the membrane preparation were incubated for 60 min at 27°C with 20 μl [^{3}H]CGP-12177 (0.4 nM) and 20 μl of incubation buffer (for total binding), KUR-1246 (0.1 nM–100 μM) or *dl*-propranolol hydrochloride (for nonspecific binding; 1 μM). Then, the medium was filtered (GF/C filter; Whatman, Maidstone, UK) and washed three times with 1 ml of ice-cold Tris-HCl buffer (75 mM; pH 7.4).

In the β3-AR binding experiment, the membrane preparation was suspended at a protein concentration of 7.5 μg/ml in incubation buffer (50 mM HEPES, 4.0 mM $MgCl_2$, and 0.04% bovine serum albumin; pH 7.5). In a test tube, 500 μl of incubation buffer containing the membrane preparation were incubated for 90 min at 37°C with 100 μl of [^{125}I]iodocyanopindolol (0.93 nM), 100 μl of atenolol (selective β1-AR antagonist; 1 μM), 100 μl of ICI-118551 (selective β2-AR antagonist; 0.1 μM), and 200 μl of incubation buffer (for total binding), KUR-1246 (0.3 μM–100 μM) or bupranolol (for nonspecific binding; 0.1 mM). Then, the medium was filtered (GF/C filter; Whatman) and washed three times with 1 ml of ice-cold Tris-HCl buffer (50 mM, pH 7.4). The radioactivity trapped on the filter was measured using a liquid scintillation counter (Packard 1900C; Downers Grove, IL) or gamma counter (Packard COBRA). The displacement of [^{3}H]CGP-12177 or [^{125}I]iodocyanopindolol binding by KUR-1246 was calculated. The inhibition constant (K_i value) for KUR-1246 was obtained by fitting the data to the following equation:

$$K_i = IC_{50}/(1 + [L]/K_d)$$

where IC_{50} is the 50% inhibitory molar concentration of the drug tested, [L] is the molar concentration of [^{3}H]CGP-12177 or [^{125}I]iodocyanopindolol present in the tube, and K_d is the dissociation constant of [^{3}H]CGP-12177 or [^{125}I]iodocyanopindolol (obtained from Scatchard plot analysis). The pK_i is the negative logarithm of K_i. The β2-AR selectivity of KUR-1246 ([IC_{50} for β1-AR]/[IC_{50} for β2-AR] or [IC_{50} for β3-AR]/[IC_{50} for β2-AR]) was calculated from three separate experiments.

In Vivo Functional Study

The experiments were carried out by the method of Kawarabayashi et al. (1996). Spontaneous uterine activity over 15-min periods was calculated by measuring the area under the intrauterine pressure curve using an integrator (NEC San-ei; 1322), and the percentage change (after versus before drug administration) was calculated. Another cannula was inserted into the carotid artery for the measurement of blood pressure and heart rate. Each drug was administered by a sequential intravenous infusion, with the dose increased every 15 min.

TABLE 1

pD_2 values for the inhibitory effects of KUR-1246 and other β2-adrenoceptor agonists on contractions of myometrium isolated from pregnant rats

Values are mean ± S.E.M. of 10 to 15 separate experiments. Numbers in parenthesis indicate potency of each drug relative to that of KUR-1246.

Drug	Myometrial Contractions			
	Spontaneous	Oxytocin-Induced	$PGF_{2\alpha}$-Induced	KCl-Induced
KUR-1246	9.04 ± 0.11 (1)	8.53 ± 0.14 (1)	7.30 ± 0.12 (1)	8.51 ± 0.09 (1)
Isoproterenol	9.44 ± 0.14* (2.27)	9.38 ± 0.06* (10.5)	8.24 ± 0.05* (7.40)	9.13 ± 0.06* (4.50)
Ritodrine	7.22 ± 0.12* (0.02)	7.12 ± 0.14* (0.04)	5.81 ± 0.14* (0.03)	6.82 ± 0.08* (0.02)
Terbutaline	7.66 ± 0.09* (0.05)	7.71 ± 0.29* (0.09)	6.24 ± 0.09* (0.10)	7.10 ± 0.14* (0.03)

* $P < 0.05$ versus the KUR-1246 group.

Statistics

The results were expressed as the mean ± S.E.M. A one-way analysis of variance was used for the statistical analysis of multiple comparisons within each group. When a significant difference was detected by one-way analysis of variance, data were further analyzed with Tukey-Kramer's test, and a P value less than 0.05 was considered to be significant.

Results

Inhibitory Effects of KUR-1246 on Myometrial Contractions in Vitro

Spontaneous and Drug-Induced Contractions of the Pregnant Rat Myometrium. Table 1 summarizes the inhibitory effects of KUR-1246 and other β-AR agonists on spontaneous and drug-evoked myometrial contractions in the pregnant rat. All the drugs tested concentration-dependently inhibited spontaneous myometrial contractions (Fig. 2). The pD_2 value for KUR-1246 was similar to that of isoproterenol. The potency of KUR-1246 was about 20 times that of terbutaline and 50 times that of ritodrine.

KUR-1246 and the other drugs also produced concentration-dependent inhibitions of the myometrial contractions elicited by oxytocin, $PGF_{2\alpha}$, and KCl. However, the potency of all four β-agonists was less against $PGF_{2\alpha}$ than oxytocin or KCl-induced contraction. The rank order of potency was isoproterenol > KUR-1246 > terbutaline > ritodrine in all experiments. The potency of KUR-1246 was 25 to 50 times that of ritodrine and 10 to 33 times that of terbutaline.

Spontaneous Contractions of the Pregnant Rabbit Myometrium. The inhibitory effects of KUR-1246 and other β-AR agonists were also evaluated in myometrial strips isolated from the pregnant rabbit. All the drugs tested elicited concentration-dependent inhibitions of both the spontaneous and oxytocin-induced contractions within the same concentration range for a given drug as observed in pregnant rat myometrium. The potency of KUR-1246 was 33 to 50 times that of ritodrine and 16 to 33 times that of terbutaline, and it was almost equal to that of isoproterenol (Table 2).

Determination of the β-AR Subtype Mediating the Inhibitory Effect of KUR-1246 on Myometrial Contractions, Using Selective β-AR Antagonists. Neither the selective β1-AR antagonist, CGP-20712A (10^{-9} to 10^{-8} M), nor the β3-AR antagonist, SR-58894A (3×10^{-9} to 3×10^{-8} M), modified the concentration-response curves for the inhibitory effect of KUR-1246 on spontaneous contractions in myometrium isolated from pregnant rats (data not shown). In contrast, ICI-118551 (3×10^{-9} to 3×10^{-8} M), the selective β2-AR antagonist, produced a parallel rightward shift in this concentration-response curve for KUR-1246 (Fig. 3A). The slope and pA_2 values obtained from the Schild plot analysis were 1.094 and 8.97, respectively, indicating a competitive antagonism by ICI-118551 against the KUR-1246-induced response (Fig. 3B).

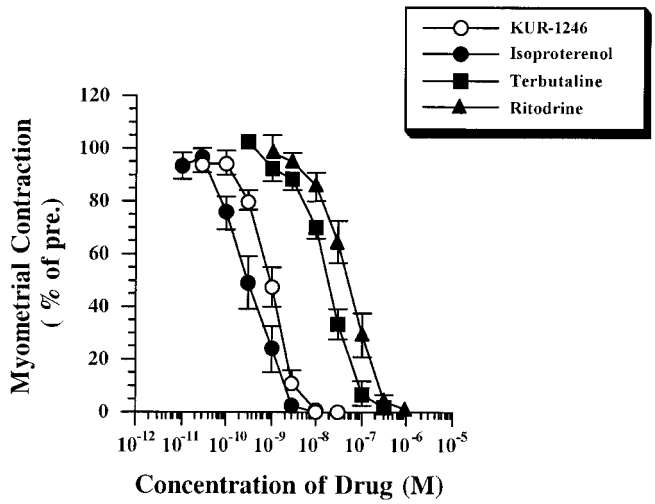

Fig. 2. Effects of KUR-1246 and other β-adrenoceptor agonists on spontaneous myometrial contractions in tissues obtained from pregnant rats on gestational day 21. Concentration-response curves for KUR-1246 (○), isoproterenol (●), ritodrine (▲), and terbutaline (■). Data represent the mean ± S.E.M. of 10 experiments.

TABLE 2

pD_2 values for the inhibitory effects of KUR-1246 and other β2-adrenoceptor agonists on contractions of myometrium isolated from pregnant rabbits

Values are mean ± S.E.M. of 8 to 10 separate experiments. Numbers in parenthesis indicate potency of each drug relative to that of KUR-1246.

Drug	Myometrial Contractions	
	Spontaneous	Oxytocin-Induced
KUR-1246	8.71 ± 0.22 (1)	8.60 ± 0.09 (1)
Isoproterenol	8.99 ± 0.11 (1.91)	8.53 ± 0.21 (0.85)
Ritodrine	6.95 ± 0.16* (0.02)	7.11 ± 0.09* (0.03)
Terbutaline	7.21 ± 0.22* (0.03)	7.39 ± 0.19* (0.06)

* $P < 0.05$ versus the KUR-1246 group.

Selectivity of KUR-1246 for β2-AR in Vitro

Functional Study Using Atria and Colon Isolated from Pregnant Rats. All drugs increased the heart rate in

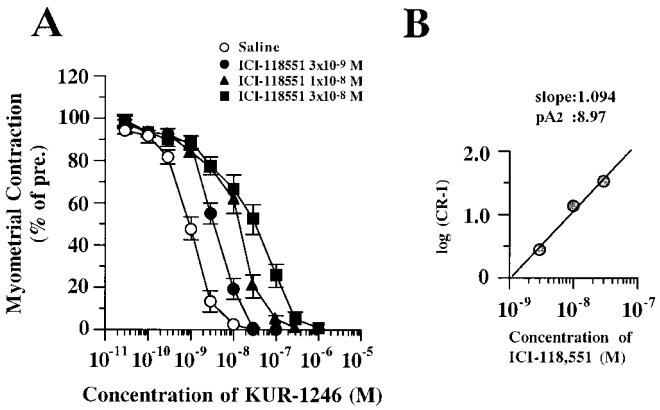

Fig. 3. Antagonism by ICI-118551 (A) of KUR-1246-induced inhibition of spontaneous contractions in myometrial strips isolated from pregnant rats on gestational day 21. ICI-118551 was added 15 min before KUR-1246 treatment. Data represent the mean ± S.E.M. of 11 to 15 experiments. (B) Corresponding Schild plot to (A).

a concentration-dependent manner. Isoproterenol was the strongest stimulant of heart rate, with its potency being about 570, 1030, and 1630 times that of ritodrine, KUR-1246, and terbutaline, respectively. With regard to the maximal positive chronotropic effects produced by these drugs, KUR-1246 produced only a 15.8% increase in rate over the basal level, which was less than that of isoproterenol (81.4%), terbutaline (81.8%), and ritodrine (54.6%). The intrinsic activity of each drug, relative to that of isoproterenol, was 0.20 for KUR-1246, 0.69 for ritodrine, and 0.96 for terbutaline (Table 3).

KUR-1246 and the other β-AR agonists each produced a concentration-dependent inhibition of the contractions of the proximal colon isolated from the pregnant rat. The IC_{50} values for these drugs is listed in Table 3. The rank order of potency was isoproterenol ≫ ritodrine ≧ KUR-1246 ≫ terbutaline.

The selectivity ratios of these drugs to inhibit spontaneously myometrial contractions versus an increase in atrial heart rate or inhibition of spontaneous colonic contractions are also summarized in Table 3. The selectivity of KUR-1246 for relaxing myometrium was 1633 and 540 compared with increasing atrial heart rate or inhibiting spontaneous contraction of the proximal colon, respectively. With regard to the selectivity of these drugs for inhibiting spontaneous myometrial contraction, the rank order of potency was KUR-1246 ≫ terbutaline > ritodrine > isoproterenol against atrial heart rate, and terbutaline > KUR-1246 > isoproterenol > ritodrine against colonic contraction.

Receptor-Binding Study Using Human β-AR. Scatchard analysis of the receptor-binding data provided K_d values of 0.4 and 0.16 nM for [^{3}H]CGP12177 binding to β1- and β2-ARs, respectively (data not shown). The K_d value obtained for [^{125}I]iodocyanopindolol binding to the β3-AR was 1.05 nM. KUR-1246 displaced radioligand binding to β1-, β2-, and β3-ARs, each in a concentration-dependent manner, with pK_i values of 5.75 ± 0.03, 7.59 ± 0.08, and 4.75 ± 0.03, respectively. The affinity of KUR-1246 for the human β2-AR was 39.2 times that for the β1-AR and 198.2 times that for the β3-AR (Table 4).

Effectiveness and Selectivity of KUR-1246 in Pregnant Rats in Vivo: Comparative Evaluation of Effects on Uterine Motility and the Cardiovascular System. Figure 4 shows a representative recording of the effect of an intravenous infusion of KUR-1246 (0.1–10.0 μg/kg/min) on spontaneous oscillation of the intrauterine pressure, heart rate, and blood pressure in the pregnant rat. KUR-1246, reduced the frequency of the spontaneous oscillation of the intrauterine pressure in a dose-dependent manner, without any detectable change in blood pressure and heart rate. The basal tone of the intrauterine pressure was also inhibited by administration of high doses of KUR-1246 (3–10 μg/kg/min). As shown in Fig. 5A, ritodrine and terbutaline as well as KUR-1246, reduced the spontaneous uterine activity with ED_{30} (30% effective dose) values of 51.29 (ritodrine), 0.76 (terbutaline), and 0.13 (KUR-1246) μg/kg/min, respectively. The inhibitory potency of KUR-1246 for this effect was about 6 times that of terbutaline, and 400 times that of ritodrine. Each of the drugs tested caused a dose-dependent increase in heart rate; however, the positive chronotropic effect of KUR-1246 was weaker than that of terbutaline and ritodrine (Fig. 5B). The maximal increase in heart rate was only 20 beats per min, even at the highest dose of KUR-1246 (10 μg/kg/min), whereas terbutaline and ritodrine produced maximal increases of about 50 to 55 beats per min over the basal level. KUR-1246, terbutaline, and ritodrine all decreased mean blood pressure (Fig. 5C). The KUR-1246-induced decrease in blood pressure was similar to that produced by terbutaline and ritodrine.

Discussion

The present study was designed to evaluate the efficacy and β2-AR selectivity of KUR-1246, a new uterine relaxant, and to compare this drug to other β2-AR agonists. The results obtained indicated that KUR-1246 is a highly selective β2-AR agonist for relaxing uterine muscle. In addition, we

TABLE 3
Selectivity of KUR-1246 and other β-adrenoceptor agonists for myometrium in pregnant rats
Numbers in parenthesis indicate the intrinsic activity of each drug relative to that of isoproterenol.

Drugs	AHR[a]	CC[b]	Selectivity for Myometrium	
			[EC_{50} to AHR]/[IC_{50} to MC][c]	[IC_{50} to CC]/[IC_{50} to MC][c]
KUR-1246	5.73 ± 0.07 (0.20)	6.31 ± 0.15	1633	540
Isoproterenol	8.71 ± 0.06* (1)	7.58 ± 0.18*	4	65
Ritodrine	5.97 ± 0.05* (0.69)	6.44 ± 0.15	14	6
Terbutaline	5.52 ± 0.06 (0.96)	4.68 ± 0.14*	117	978

* $P < 0.05$ versus the KUR-1246 group.
[a] Positive chronotropic effects on atrial heart rate (AHR).
[b] Inhibition of spontaneous colonic contractions (CC).
[c] The IC_{50} values for spontaneous myometrial contractions (MC) were taken from Table 1.

TABLE 4

Inhibition constant (pK_i) and β2-adrenoceptor selectivity of KUR-1246 for the displacement of [^{3}H]CGP12177 or [^{125}I]iodocyanopindolol-binding to membrane preparations expressing human β1-, β2-, and β3-adrenoceptors

The pK_i values are the mean ± S.E.M. of three separate experiments.

Drug	β-Adrenoceptor Subtype (pK_i)			β2-Adrenoceptor Selectivity	
	β1	β2	β3	[IC_{50} for β1]/[IC_{50} for β2]	[IC_{50} for β3]/[IC_{50} for β2]
KUR-1246	5.75 ± 0.03	7.59 ± 0.08	4.75 ± 0.03	39.2	198.2

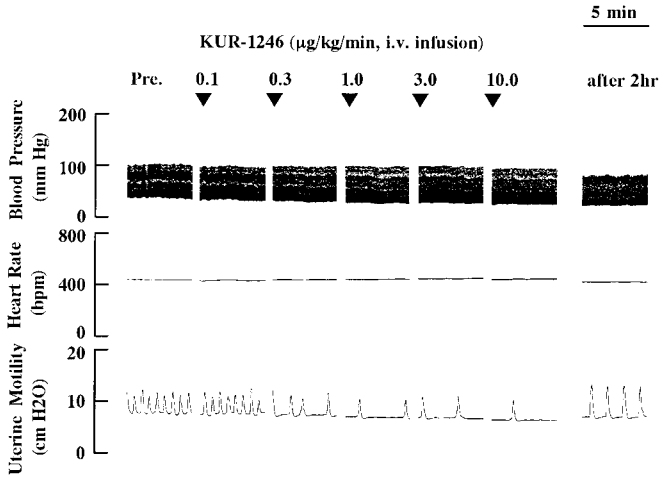

Fig. 4. Representative recording of the effects of KUR-1246 on intrauterine pressure, blood pressure, and heart rate in an anesthetized pregnant rat on gestational day 21.

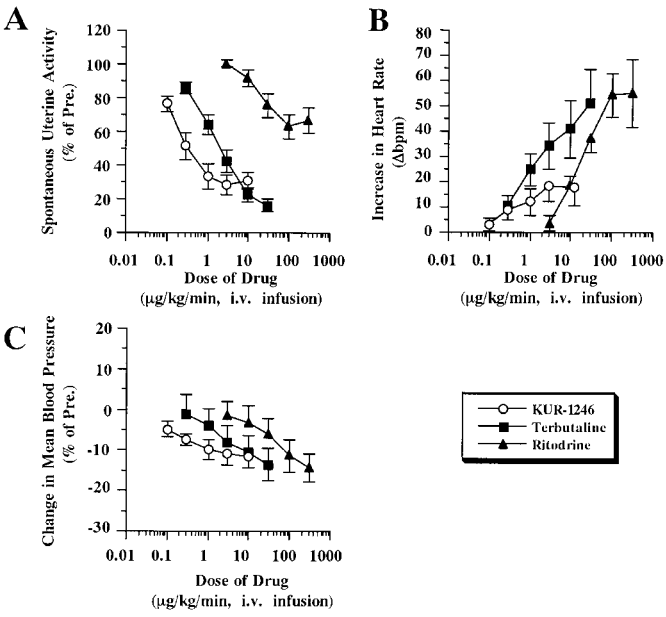

Fig. 5. Effects of KUR-1246 (○), terbutaline (▲), and ritodrine (■) on spontaneous uterine activity (A), heart rate (B), and change in mean blood pressure (C) in anesthetized pregnant rats on gestational day 21. Each drug was administered by sequential intravenous infusion, the dose being increased every 15 min. The ED_{30} (30% effective dose) values were: KUR-1246, 0.13 μg/kg/min; terbutaline, 0.76 μg/kg/min; and ritodrine, 51.29 μg/kg/min. Values are mean ± S.E.M. of 10 experiments.

have previously found by using receptor-binding assays that, KUR-1246 did not bind to other receptors, except for β-ARs, including α-adrenergic, histamine, muscarine, and dopamine receptors (data not shown).

It is well known that rodent studies estimating the efficacy of β2-AR agonists have been good predictors of the clinical utility of these drugs. In our experiments, we compared the inhibitory effects of KUR-1246 and other β-AR agonists on spontaneous or drug-induced contractions of myometrium isolated from pregnant rats and rabbits. KUR-1246 inhibited both types of myometrial contraction in both species, and it was the most potent myometrial relaxant among the β-AR agonists clinically used as tocolytics. However, all the β-AR agonists tested exhibited a significantly lower inhibitory potency (one-tenth or less) for $PGF_{2\alpha}$-induced myometrial contractions than for oxytocin- and KCl-induced contractions. A number of investigators have reported that oxytocin and $PGF_{2\alpha}$ contribute to myometrial contractions in preterm labor (Romero et al., 1989; Kobayashi et al., 1999). An increase in the intracellular Ca^{2+} concentration plays an essential role in the generation of smooth muscle contractions. An influx of extracellular Ca^{2+} into the intracellular space via voltage-dependent Ca^{2+} channels and the subsequent release of Ca^{2+} from intracellular sites may be both involved in this process. Activation of voltage-dependent Ca^{2+} channels is thought to be mainly responsible for the generation of both spontaneous and KCl-induced myometrial contractions, whereas release of Ca^{2+} from intracellular sites is also involved in the increase in the intracellular Ca^{2+} concentration underlying oxytocin-induced contractions (Kawarabayashi et al., 1997). In contrast, $PGF_{2\alpha}$ produces myometrial contractions primarily by stimulating intracellular sites to release Ca^{2+} (Davis et al., 1987; Yang et al., 1997). It is well established that β-AR agonists act by hyperpolarizing the myometrial plasma membrane, thus interfering with the influx of extracellular Ca^{2+} (Huszar and Walsh, 1989). On this basis, it seems most likely that the lower potency shown by the β-AR agonists against $PGF_{2\alpha}$-induced contractions might reflect differences in the site of action between β-AR agonists and $PGF_{2\alpha}$. KUR-1246 and the other β-AR agonists all completely inhibited $PGF_{2\alpha}$-induced contractions when their concentration was increased further. Since β-AR agonists, at high concentrations, also facilitate incorporation of intracellular Ca^{2+} into storage sites, it may be possible to use KUR-1246 effectively against $PGF_{2\alpha}$-related myometrial contractions if it indeed has a higher selectivity for the uterus than for other tissues.

Therefore, we analyzed the mode of action of KUR-1246 by using specific β-AR antagonists. Neither CGP-20712A nor SR-58894A, at concentrations that occupy virtually all β1- or β3-AR (Kaumann, 1986; Manara et al., 1995), antagonized the relaxing effect of KUR-1246 on the pregnant rat myometrium. On the other hand, ICI-118551, a selective β2-AR antagonist, produced an effective competitive antagonism against the KUR-1246-induced inhibition of myometrial contractions. The pA_2 value and the slope of the Schild plot for ICI-118551 are quite similar to those reported by Bilski et al. (1983). These data clearly demonstrate that KUR-1246 produced its inhibitory effect on myometrial contractions by

stimulating the β2-AR subtype. With regard to the β3-AR, there are as yet no reports in the literature supporting a possible functional role in the myometrium, although many species, including the rat and human, have been examined. Our experiments provided no evidence in favor of a functional β3-AR in the myometrium in the pregnant rat.

We examined the β2-AR selectivity of KUR-1246 and other β-AR agonists in two sets of experiments: namely, functional and receptor-binding experiments. In the functional experiment, using myometrium, atria, and proximal colon, isolated from the pregnant rat, our main aim was to compare the inhibitory effect of KUR-1246 on the myometrium with its effects on the other tissues. All the drugs tested increased the heart rate (β1-AR-mediated response), but the maximal positive chronotropic effect of KUR-1246 was definitely much lower than that of the other three drugs, indicating that KUR-1246 acted on the rat atria only as a partial agonist. This is one of the most important pharmacological characteristics of KUR-1246 that suggest that it has potential as a clinically beneficial drug with few side effects on the heart. Additional studies will be needed to clarify the behavior of KUR-1246 as a partial agonist on β1-AR. KUR-1246 also inhibited spontaneous contractions of the isolated rat colon in the presence of both specific β1-AR- and β2-AR antagonists. But its potency was very low, compared with that of isoproterenol (one-thousandth). Since β3-AR mediates such responses as thermogenesis (Goldberg and Frishman, 1995), lipolysis (Umekawa et al., 1999), and relaxation of the colon (Ponti et al., 1996) in humans, this low potency should be another benefit of KUR-1246 in the treatment of pregnant women. This functional experiment clearly demonstrated that KUR-1246 has excellent selectivity for the myometrial β2-AR over both β1-AR (1630 times) and β3-AR (540 times).

In the receptor-binding assay, we used membrane preparations obtained from cells expressing each of the cloned human β-AR subtypes. The affinity of KUR-1246 for the human β2-AR was higher than that for β1-AR (39.2 times) and β3-AR (198.2 times). However, the selectivity ratios were somewhat lower in the binding assay than in the functional assay, possibly a reflection of the different methods and/or species used. Indeed, other researchers have reported that a given compound does not necessarily produce an identical result in a binding assay and a functional assay (Maguire et al., 1976; Minneman et al., 1981; Ohashi et al., 1996).

In the last in vivo experiment, we evaluated both the efficacy and selectivity of KUR-1246 to try to confirm the results obtained in the functional in vitro experiment, and we made comparisons with ritodrine and terbutaline. KUR-1246 strongly suppressed spontaneous uterine contractions without causing severe cardiovascular side effects, such as tachycardia and hypotension. These results were fully in accord with those obtained in the in vitro experiments. Thus, we confirmed the potent and selective inhibitory effects of KUR-1246 on uterine contractions in the pregnant rat in vivo.

In conclusion, we have clearly demonstrated in the present study that KUR-1246 is a potent and highly selective β2-AR agonist. On the basis of the evidence available so far, it shows great potential as a drug for the treatment of preterm labor in humans.

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