Use of a novel and highly selective oxytocin receptor antagonist to characterize uterine contractions in the rat

Gerald P. McCafferty,¹ Mark A. Pullen,¹ Charlene Wu,¹ Richard M. Edwards,¹ Michael J. Allen,³ Patrick M. Woollard,² Alan D. Borthwick,² John Liddle,² Deirdre M. B. Hickey,² David P. Brooks,¹ and Timothy D. Westfall¹

¹Departments of Urogenital Biology, ²Medicinal Chemistry and Drug Metabolism and Pharmacokinetics, Cardiovascular and Urogenital Center of Excellence for Drug Discovery, GlaxoSmithKline Research and Development, King of Prussia, Pennsylvania and Gunnels Wood Road, Stevenage, Herts, United Kingdom; and ³Department of Assay Development and Compound Profiling, GlaxoSmithKline, New Frontiers, Science Park, Harlow, Essex, United Kingdom

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PRETERM BIRTH, WHICH IS DEFINED as delivery before 37 wk, accounts for between 8 and 12% of all births and is the leading cause of infant morbidity and mortality (16). There is an increase in neonatal survival and a substantial reduction in morbidity associated with increasing gestational age (15). Delaying delivery by 48 h allows physicians to administer a full course of glucocorticoids to the expectant mother, which can reduce the incidence of respiratory distress syndrome in the preterm neonate (7, 24). However, there is a need for agents that can prolong gestation for longer periods of time and allow the pregnancy to progress closer to term. Various tocolytics such as β2-adrenoceptor agonists, prostaglandin synthase inhibitors and calcium channel antagonists have shown some efficacy in treating preterm labor; however, they are often prematurely discontinued due to maternal and fetal side effects (14). Atosiban, a peptide antagonist of oxytocin and vasopressin V1a receptors, has been approved for use in Europe. It has been shown to reduce uterine contractions in vitro and in vivo and arrest preterm labor in pregnant women, resulting in a delay of delivery (13). While atosiban is efficacious at delaying delivery for 48 h, its use to prolong pregnancy beyond 48 h has been limited because it is only available for parenteral administration.

Significant progress has been made in identifying both peptide and nonpeptide oxytocin receptor antagonists (1, 6, 20, 25, 27, 31, 32, 37). However, even though the newly developed peptide antagonists [such as barusiban and those described by Manning et al. (20) and Stymiest et al. (32)] are more potent and selective and serve as good research tools, their use in the clinic is still limited by having to be administered parenterally. Some of the early efforts to produce orally acting nonpeptide antagonists suffered from a lack of bioavailability such as L-371,257 (16). More recent efforts have produced compounds that have better bioavailability, but selectivity over vasopressin receptors can still be improved (2). In the present study, we describe a novel, selective and bioavailable oxytocin receptor antagonist that inhibits oxytocin-induced and spontaneous uterine contractions in vitro and in vivo. Preliminary data were reported at the APS Conference in Steamboat Springs, CO (4).

MATERIALS AND METHODS

In Vitro Experiments

Membrane preparations. Chinese hamster ovary (CHO) cells were stably transfected with human oxytocin, V1a, V1b, and V2 receptors using internal ribosome entry point transfection (26, 39). Membranes were prepared from cells cultured in 1,800 cm² roller bottles as follows. The cells were harvested with HBSS + 0.6 mM EDTA and spun down at 250 g for 5 min at 4°C. This was repeated after resuspending the pellets in 200 ml HBSS + 0.6 mM EDTA. All subsequent steps were performed at 4°C. The cells were homogenized within a glass Waring blender for 2 × 15 s in 200 ml of 50 mM HEPES + 10 μM leupeptin + 25 μg/ml bacitracin + 1 mM EDTA + 1 mM PMSF + 2 μM Peptatin A, (the latter two reagents were added as fresh × 100 and × 500 stocks, respectively, in ethanol). The membranes were then washed at 4°C with 10 mM HEPES pH 7.4, 1 mM EDTA, and 1% Triton X-100.

Address for reprint requests and other correspondence: T. D. Westfall, 709 Swedeland Rd., Mail Code UW2521, King of Prussia, PA 19406, USA (e-mail: timothy.d.westfall@gsk.com).

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blender was plunged into ice for 5 min after the first burst and 10–40 min after the final burst to allow foam to dissipate. The material was then spun at 500 g for 15 min, and the pellet was resuspended in buffer containing 50 mM Tris, pH 7.5, 0.25 M sucrose, 5 mM MgCl₂, 1 mM EDTA, and 0.1 µM/ml aprotinin. The cell lysate was homogenized 30 times using a glass dounce homogenizer and centrifuged at 800 g for 10 min to remove unbroken cells and nuclei. The supernatant was centrifuged at 40,000 g for 15 min, and the pellet was resuspended in buffer containing 50 mM Tris, pH 7.5, 0.25 M sucrose, 5 mM MgCl₂, 1 mM EDTA, and 100 µg/ml bacitracin. Small aliquots were stored at −70°C. Rat kidney and liver tissue were homogenized (1 g/10 ml) in the same hypotonic lysis buffer with a motor-driven Teflon homogenizer (20 strokes) and processed the same way as above.

Binding studies. Saturation binding experiments of [³H]vasopressin or [³H]oxytocin to rat tissue, HEK cell membranes expressing rat oxytocin, CHO cell membranes expressing human OT receptors or human V1a, V1b, or V2 receptors were performed as described by Wyatt et al. (39) with modification. Briefly, 0.1 nM to 3 nM of radioligands were added to membranes in duplicate in the absence (total binding) or presence (nonspecific binding) of unlabeled ligands (1 µM) in 200 µl assay buffer (50 mM Tris, pH 7.5, 10 mM MgCl₂ and 0.1% BSA) and incubated for 60 min at room temperature. After the incubation, the reactions were stopped with 3 ml ice-cold buffer (120 mM NaCl, 4.7 KCl, 1.2 K₂HPO₄, 2.5 CaCl₂, 1.2 MgSO₄, 25 NaHCO₃, and 11 glucose) and aerated with 95% O₂ and 5% CO₂. The myometrial strips were equilibrated at 37°C under 1 g of passive tension until a stable baseline was achieved (90 min). Krebs solution was changed every 15 min during the equilibration period. The muscle tension was recorded by an isometric force transducer (Grass FT 03; Grass Instruments, Quincy, MA); the electrical signal for tension was recorded using a BIOPAC MP150 acquisition unit connected to a desktop computer using AcqKnowledge version 3.7.0 software (BIOPAC Systems Inc, Goleta, CA). At the end of the equilibration period and after a 30 min pretreatment with the antagonist or vehicle, a cumulative concentration-response curve to oxytocin was conducted with 5-min intervals between 1/2 log additions of oxytocin from 10⁻¹¹ to 10⁻⁵ M. Oxytocin contractile activity was defined as the integral of the tension-time curve [area under the curve (AUC)] for 5 min minus the basal AUC before the addition of oxytocin. The pA₂, EC₅₀ (concentration-response = 50% of the maximal response), and maximal concentrations were calculated from the concentration-response curves in the absence and presence of antagonist using nonlinear regression (GraphPad Prism Software ver. 3.02; GraphPad Prism, San Diego, CA).

In Vivo Experiments

Uterine contractions in anesthetized nulliparous rats. Nulliparous female Sprague-Dawley (240–320 g) rats were treated with DES 18 h before the experiment. Rats were surgically prepared as described in Wyatt et al. (38). On the day of the experiment, the rats were anesthetized with 60 mg/kg ip pentobarbital sodium and placed on a heating pad to maintain body temperature. A maintenance intravenous infusion of 10 mg·kg⁻¹·h⁻¹ (dose volume = 0.1 ml/h) pentobarbital sodium commenced 2 h postinduction, and the rate was intermittently increased when required. The trachea was cannulated, with polyethylene (PE)-240 (ID 1.67, OD 2.42 mm; Becton Dickinson, NJ) to allow spontaneous respiration. Both femoral arteries and veins were cannulated with tygon plastic tubing (ID 0.508, OD 1.016 mm) to monitor blood pressure, heart rate, blood sampling and intravenous administration of drugs, respectively. A midline abdominal incision was made, and the left uterine horn was exposed. Silk suture was tied around the anterior end of the uterine horn (1 cm posterior to the ovary) and a second positioned 0–1 cm posterior to the first tie. The anterior end of the uterine horn was anchored in the abdominal cavity, and the posterior end was connected to a Grass FT 03 isometric force transducer under a resting tension of 2 g. After surgery was complete, the rats were allowed to stabilize, during which the tension on the uterine horn was readjusted to 2 g. The exposed uterine horn was irrigated with 0.5 ml of warm saline every 10–15 min throughout the experiment. Blood pressure and heart rate were monitored using a pressure transducer (Gould-Statham P23XL). The electrical signals for blood pressure, heart rate, and uterine contrac-
obtained with the second control oxytocin challenge. The ID₅₀ (that each oxytocin injection was calculated compared with the AUC the oxytocin injection. The AUC post-GSK221149A for 10 min after 10 min minus a 2-min AUC sample normalized to 10 min before the oxytocin injection. The AUC post-GSK221149A for 10 min after each oxytocin injection was calculated compared with the AUC obtained with the second control oxytocin challenge. The ID₅₀ (that dose that reduced the control response by 50%) was calculated from the dose-response curve using linear regression. Five experiments were performed using cumulative doses of GSK221149A (0.1, 0.3, and 1.0 mg/kg iv). Two-hundred-microliter blood samples were taken 25 min after each dose of GSK221149A. A final blood sample was drawn 85 min after the last intravenous administration of GSK221149A. Rats were administered 200 μl of saline intravenously after blood samples were taken. The blood samples were centrifuged at 14,000 rpm for 2 min; the plasma was collected and frozen at −20°C.

In a separate study, the effect of oral dosing with GSK221149A (after 1 and 4 days dosing) on oxytocin-induced uterine contractions was assessed. Rats were orally dosed with 5 mg/kg GSK221149A (or vehicle) for 1 or 4 days (once a day, UID) and prepared as above. Uterine contractile activity induced by oxytocin (0.3 μg/kg iv) was determined 2.5 h after oral administration of GSK221149A or vehicle.

**Uterine contractions in anesthetized late-term pregnant rats.** Pregnant rats (19–21 days gestation; normal gestational age at delivery is 22 days) were anesthetized with isoflurane (4% for induction) and treated with 60 mg/kg ip pentobarbital sodium for surgery. The animals were surgically prepared as above for the administration of drug, blood sampling, and monitoring of blood pressure. Changes in uterine tension were used to record uterine contractility in the non-pregnant rat studies described previously (both in vivo and in vitro). However, measuring uterine tension in pregnant rats proved challenging, and we were able to modify the protocol to record uterine pressure instead. Therefore, a midline abdominal incision was made, the left uterine horn was exposed, and its tubule end (near the ovary) was ligated with surgical silk. The uterine wall was incised and PE-60 (ID 0.76, OD 1.22 mm Becton Dickinson, Franklin Lakes NJ) tubing fitted with a liquid-filled plastic film balloon tied to the end of the catheter (1 cm, S.C. Johnson & Son, Racine, WI) was inserted into the lumen and secured to the uterine wall with a purse-string silk suture. The balloon was inflated with 0.1 ml of saline and connected to a pressure transducer (Gould-Statham P23XL). Intraperitoneal pressure, blood pressure, and heart rate were recorded digitally using a BIOPAC MP150 (BIOPAC Systems Inc, Goleta, CA) acquisition unit connected to a desktop PC using AcqKnowledge Waveform Data Analysis software ver. 3.7.0 (BIOPAC Systems). After a 40 to 50-min stabilization period, a bolus dose of oxytocin (0.3 μg/kg iv) was administered to ensure the rat would respond to oxytocin. A further five doses of oxytocin were administered at 5 min intervals: a second control response, 3 responses 15 min post increasing doses of GSK221149A and 1 recovery response was elicited. Oxytocin-induced uterine activity was calculated by measuring the integral under the time-tension curve (AUC) at 10 min after each oxytocin injection.

**Statistics**

Values in the text refer to means ± SE. Statistical significance was determined by repeated-measures ANOVA with Tukey’s multiple comparison test or Student’s paired t-test where appropriate. Differences were considered significant when P < 0.05.

**Drugs and Materials**

GSK221149A (3R,6R)-3-Indan-2-yl-1-[(1R)-1-(2-methyl-1,3-oxazol-4-yl)-2-morpholin-4-yl-2-oxoethyl]-6-[(1S)-1-methylpropyl]-2,5-piperazinedione was synthesized at GlaxoSmithKline (Stevenage, UK). The following chemicals were obtained from commercial sources: Diethylstilbestrol, DMSO, polyethylene glycol 200 (PEG200), corn oil, oxytocin, indomethacin (Sigma, St. Louis, MO); Nembutal Sodium Solution (pentobarbital sodium), Isofllo (isoflurane) (Abbott, Chicago IL), atosiban 1-(3-mercapto propanoic acid)-2-(O-ethyl-N-tosyl)-4-L-threonine-8-L-ornithine-oxytocin (Ferring), [³H]Hypoxaspressin and [³H]Oxytocin (PerkinElmer, Waltham, MA). For in vitro experiments, GSK221149A was dissolved in DMSO as a 10 mM stock solution (serial dilutions were made in DMSO). GSK221149A was dissolved in 20% DMSO 30% PEG200 and 50% water for intravenous administration and kept frozen for no more than 1 wk. For oral administration, GSK221149A was suspended in 1% hydroxypropyl methyl cellulose (HPMC) 50% water and made fresh daily. Oxytocin was dissolved in water and stored frozen at −20°C until the day of the experiment and diluted with saline for intravenous administration.

**RESULTS**

Competitive binding assays using [³H]Oxytocin were carried out to determine the binding affinity of GSK221149A (Fig. 1) using liquid chromatography/tandem mass spectrometry (LC/MS/MS) detection. GSK221149A was isolated from 50 μl of rat plasma using a protein precipitation method by addition of 200 μl of 95% acetonitrile/5% 10-mM ammonium formate (vol/vol; pH 3.0) containing 200 ng/ml of a proprietary structural analog of GSK221149A as an mass spectral internal standard; the mixture was vortex-mixed and centrifuged for 40 min at 4,000 rpm. One-half microliter of the resulting supernatant was injected onto the LC/MS/MS system using a HPLC PAL (liquid introduction system for high throughput analysis) autosampler (CTC Analytics, Zwingen, Switzerland) coupled to a Rhos 2000 pump (Flux Instruments, Basel, Switzerland). The mobile phase consisted of an isocratic flow of 70% acetonitrile and 30% 10 mM ammonium formate (pH 3.0), while the HPLC column was a 2.0 × 50 mm, 5 μm, Pursuit C18 (Varian, Palo Alto, CA). The eluent flowed into a Sciex API4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) using positive ion atmospheric pressure chemical ionization and operated under multiple-reaction monitoring conditions. GSK221149A was characterized by the transition of the parent precursor ion to its product ion, generated at a collision energy setting of 25 V, and the proprietary internal standard was characterized by the transition of the precursor ion to the product ion, generated at a collision energy setting of 36 V.

Data were determined as quantitative drug concentrations as determined by standard calibration curve analysis, using linear fitting (r = 0.9991) of a 1/x-weighted plot of the GSK221149A/internal standard peak area ratios vs. GSK221149A concentration, with a linear analytical range of 10.0 to 10,000 ng/ml. A plasma IC₅₀ was determined by plotting contractile response (expressed as percentage control) vs. plasma concentration.

**fig. 1.** Structure of GSK221149A (3R,6R)-3-Indan-2-yl-1-[(1R)-1-(2-methyl-1,3-oxazol-4-yl)-2-morpholin-4-yl-2-oxoethyl]-6-[(1S)-1-methylpropyl]-2,5-piperazinedione.
and atosiban to recombinant oxytocin receptors from rat and human. GSK221149A was found to have nanomolar affinity for oxytocin receptors from both species with 10-fold greater affinity for the human vs. the rat receptors (Table 1). Competitive binding assays using \(^{3}H\)vasopressin demonstrated that GSK221149A was highly selective for the human oxytocin receptor vs. human recombinant V1a (\(>18,000\)-fold), V1b (\(>15,000\)-fold) and V2 (\(>1,400\)-fold) receptors. In addition, GSK221149A was found to be highly selective for rat recombinant oxytocin receptors vs. native V1 (\(>1,500\)-fold) and V2 (\(>400\)-fold) receptors. Atosiban also had nanomolar affinity for rat and human oxytocin receptors but was much less selective vs. vasopressin receptors. Atosiban was found to be \(~70\)-fold more potent at human recombinant V1a receptors than at human OT receptors and was only 10-fold and 30-fold selective for human OT vs. human V1b and V2 receptors. A similar lack of selectivity was observed with atosiban at rat receptors. It was equipotent at rat OT and V2 receptors and 10-fold selective for rat OT vs. rat V1 receptors (Table 1). The selectivity of atosiban was very similar to that reported in the literature by various groups (6, 20, 36). For example, Cirillo et al. (6) found that atosiban was 23-fold more potent at hV1a than hOT and only 8 and 12-fold more potent at hOT than V1b and V2, respectively.

GSK221149A was found to have no measurable agonist activity at human or rat recombinant OT receptors or human recombinant V1a or V1b receptors in the FLIPR calcium assay up to concentrations of 10 \(\mu\)M (not shown). GSK221149A was found to produce a functional antagonism of human and rat oxytocin receptors with functional \(K_{is}\) of 5.9 and 53 nM, respectively (Table 2). GSK221149A had no measurable antagonist activity at human V1a or V1b receptors.

GSK221149A (10\(^{-7}\) to 10\(^{-5}\) M) produced parallel rightward shifts of oxytocin-induced concentration-response curves in rat isolated myometrial strips (Fig. 2A). The slope and \(pA_{2}\) value obtained from Schild plot analysis were 0.947 and 8.18, respectively, indicating a competitive antagonism by GSK221149A (Fig. 2B).

Table 1. Binding affinity of GSK221149A and atosiban for oxytocin and vasopressin receptors from humans and rats

<table>
<thead>
<tr>
<th>Receptor</th>
<th>GSK221149A Ki, nM</th>
<th>Atosiban Ki, nM</th>
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<tbody>
<tr>
<td>hOT</td>
<td>0.65 ± 0.14</td>
<td>11 ± 0.7</td>
</tr>
<tr>
<td>hV1a</td>
<td>&gt;12,000</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>hV1b</td>
<td>&gt;10,000</td>
<td>44 ± 11</td>
</tr>
<tr>
<td>hV2</td>
<td>950 ± 80</td>
<td>330 ± 34</td>
</tr>
<tr>
<td>Rat OT</td>
<td>4.1 ± 0.82</td>
<td>32 ± 5.0</td>
</tr>
<tr>
<td>Rat V1 (liver)</td>
<td>6,200 ± 3,120</td>
<td>310 ± 14</td>
</tr>
<tr>
<td>Rat V2 (kidney)</td>
<td>1,630 ± 150</td>
<td>29 ± 2.8</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE, OT, oxytocin; GSK221149A, (3R,6R)-3-Indan-2-yl-1-[(1R)-1-(2-methyl-1,3-oxazol-4-yl)-2-morpholin-4-yl-2-oxoethyl]-6-[(1S)-1-methylpropyl]-2,5-piperazinedione.

Intravenous administration of GSK221149A (0.1 to 1.0 mg/kg) resulted in a dose-dependent reduction in oxytocin-induced uterine contractions in anesthetized rats (Fig. 3). Both the 0.3 and 1.0 mg/kg doses of GSK221149A produced statis-

Table 2. Functional antagonist potency of GSK221149A at oxytocin and vasopressin receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>GSK221149A Functional Ki, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>hOT</td>
<td>5.9</td>
</tr>
<tr>
<td>hV1a</td>
<td>&gt;680</td>
</tr>
<tr>
<td>hV1b</td>
<td>&gt;2,500</td>
</tr>
<tr>
<td>Rat OT</td>
<td>53</td>
</tr>
</tbody>
</table>

Fig. 2. A: effect of GSK221149A on oxytocin-induced contractions in rat myometrial strips. B: corresponding Schild plot. Data represent the means ± SE (n = 4).

Fig. 3. Effect of GSK221149A (iv) on oxytocin-induced uterine contractions in anesthetized rats. Data represent means ± SE (n = 5). **p < 0.01, ***p < 0.001 vs. control with repeated-measures ANOVA with Tukey’s post hoc test. ID\(_{50}\) = 0.27 ± 0.06 mg/kg iv. AUC, area under the curve.
tically significant reductions in oxytocin-induced contractions of 62% and 94%, respectively. Eighty-five minutes after the 1.0 mg/kg dose, oxytocin-induced uterine contractions were still significantly inhibited by 63%. Plasma was taken 25 min after each dose of GSK221149A and analyzed for compound (Table 3). The plasma concentration that corresponded to 50% inhibition of the contraction induced by oxytocin was 88 ng/ml (Fig. 5). There was no statistically significant change in blood pressure or heart rate observed with any dose of GSK221149A (data not shown).

Oral administration of GSK221149A (5 mg/kg) to rats significantly blocked oxytocin-induced uterine contractions by 72% (Fig. 4). A similar inhibition (60%) of oxytocin-induced contractions was observed after 4 days of dosing with GSK221149A (5 mg/kg po UID). In addition, a similar relationship between plasma concentration and contractile response was observed after oral dosing, as was observed after intravenous dosing (Fig. 5). No significant changes in blood pressure and heart rate compared with corresponding controls were measured in rats treated orally with 5 mg/kg of GSK221149A UID for 1 or 4 days.

Intravenous administration of GSK221149A to late-term pregnant rats significantly reduced spontaneous uterine contractions in a dose-dependent manner (Fig. 6). Doses of 0.3, 1, and 3 mg/kg of GSK221149A decreased spontaneous contractions by 25.7 ± 2.8%, 33.1 ± 19.5%, and 44.1 ± 14.6%, respectively. No significant changes in blood pressure and heart rate were observed with vehicle or GSK221149A treatment.

DISCUSSION

In the present study, we have demonstrated that a novel oxytocin receptor antagonist that is highly selective over the closely related vasopressin receptors (V1a, V1b, and V2), reduces uterine contractility in the rat. There is compelling evidence that oxytocin plays an important role in both term and preterm labor. Oxytocin causes contraction of myometrial smooth muscle; indeed, oxytocin (Pitocin) is used in the clinic to induce labor. Plasma oxytocin is increased during labor (11, 35), and myometrial oxytocin receptor density increases during pregnancy, peaking in labor in both rats and humans (9, 17). Uterine contractile activity and responsiveness to oxytocin in both rats and humans correlate with oxytocin receptor density (10, 19), and failure to respond to induction of oxytocin is associated with a reduced concentration of oxytocin receptors in humans (28). There is also growing evidence that the oxytocin gene is expressed and the peptide is synthesized in intrathecal tissues (3, 5, 33). Oxytocin receptor antagonists

<table>
<thead>
<tr>
<th>Blood Sample No.</th>
<th>Dose, mg/kg iv</th>
<th>Time Post-injection, min</th>
<th>Concentration, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>25</td>
<td>47 ± 8</td>
</tr>
<tr>
<td>2</td>
<td>0.3</td>
<td>25</td>
<td>138 ± 25</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>25</td>
<td>512 ± 70</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>85</td>
<td>193 ± 43</td>
</tr>
</tbody>
</table>

Values represent the means ± SE of 5 experiments.

Fig. 4. Effect of oral administration of GSK221149A on oxytocin-induced uterine contractions in anesthetized rats. GSK221149A was administered once a day for 1 and 4 days. Oxytocin (0.3 μg/kg iv) was administered 2.5 h after the last treatment with vehicle or GSK221149A. Values represent the means ± SE (n = 4 or 5). ***P < 0.001 vs. corresponding control with ANOVA with Tukey’s post hoc test.

Fig. 6. Effect of intravenous administration of GSK221149A on spontaneous uterine contractions in anesthetized late-term pregnant rats. Responses are expressed as a percentage change of the time-tension curve for 10 min before and 15 min after treatment. Each bar represents the mean ± SE (n = 4 or 5). **P < 0.01, ***P < 0.001.
have been shown to inhibit spontaneous myometrial contractions in vitro (36) and suppress myometrial activity in animal models in vivo (23, 25). Furthermore, in clinical studies, oxytocin receptor antagonists have been shown to inhibit uterine contractions in pregnant women (12) and in women during the postpartum period (8). The peptide atosiban, a mixed oxytocin-vasopressin receptor antagonist, has been reported to be as effective as beta-adrenergic agonists in delaying delivery for 24 h but with fewer side effects (22). In a placebo-controlled study (30), the percentage of patients undeivered and that did not require an alternate tocolytic therapy was greater in the atosiban group than in the placebo group. While the duration of therapy (48 h) was sufficient to permit administration of steroids to aid lung development, it is likely not to have been long enough to clearly demonstrate an improvement in neonatal outcomes. Neonatal mortality was significantly increased in the atosiban group (<24 wk gestation) compared with the placebo group. This may have been due, in part, to unequal randomization such that there were significantly more women at extremely early gestation randomized to the atosiban group compared with the placebo group. It has, however, been speculated that the vasopressin antagonist activity of atosiban may have been detrimental. Atosiban actually has a higher affinity for some vasopressin receptor subtypes than it has for oxytocin receptors. Our data indicate that it is 70 times more potent at the V1a receptor than at the oxytocin receptor. Blockade of the V1a receptor would only be expected to have physiological effects in situations where vasopressin is supporting blood pressure (such as following hemorrhage). Blockade of the V1b receptor, however, might be expected to block the compensatory responses to stress since the V1b receptor mediates ACTH release (34). Since V2 receptors have been shown to be active in the fetus of some species, blockade of V2 receptors might be expected to cause diabetes insipidus in the neonate and polyhydrannous, since neonatal urine is the source of amniotic fluid (18, 29). There are no published data available on amniotic fluid volumes in atosiban-treated patients. However, atosiban is unlikely to produce polyuria in humans since the affinity of atosiban for the V2 receptor is significantly less than that for the V1a, V1b, or oxytocin receptors. The role of vasopressin receptor antagonism in the treatment of preterm labor remains an open question. It is possible that vasopressin antagonist activity may confer an advantage with regard to efficacy over a selective oxytocin receptor antagonist. Despite some evidence to suggest that vasopressin is uterotonic (19), there is little other supportive evidence for a major role of vasopressin in preterm labor. Plasma vasopressin levels do not appear to increase during labor (21), and there is little change in vasopressin receptor density during pregnancy or labor (19). If vasopressin receptor antagonist activity does not contribute to uterine quiescence and in fact is detrimental, the exquisite selectivity of GSK221149A for the oxytocin receptor might be expected to provide an advantage over atosiban.

Peptide antagonists that are more potent and selective than atosiban have been identified. For example, those described by both Manning et al. (20) and Stymiest et al. (32) are reported to be as potent as GSK221149A. However, the most selective peptide described by Manning et al. (20) is only 6.5-fold selective for human OT vs. human V1a receptors, and no selectivity data are reported by Stymiest et al. (32). Recently discovered nonpeptide antagonists are reported to have much better selectivity for OTR vs. V1aR; however, none approach the selectivity of GSK221149A. For example, the compounds described by Cirillo et al. (6), Serradeil-Le Gal et al. (31) (SSR126768A), and Bell et al. (2) (L-372,662) are 6-, 95-, and 609-fold selective for hOTR vs. hV1aR, while GSK221149A is >18,000-fold selective. In addition, GSK221149A (1,500-fold) is much more selective than SSR126768A (62-fold) at rat OTR vs. rat V1a (data for the other two previously mentioned nonpeptide antagonists are lacking).

In summary, GSK221149A displays excellent selectivity for both rat and human oxytocin receptors. Both spontaneous and induced uterine contractions in the rat were found to be sensitive to inhibition by GSK221149A. These findings provide evidence that a selective orally active oxytocin antagonist, such as GSK221149A may have the potential for safely delaying labor significantly longer than 48 h, perhaps providing a significant improvement in neonatal outcomes.

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