In-vivo and in-vitro assessment of the influence of ritodrine and oxytocin on the placental secretion of human choriionic gonadotrophin and placental lactogen

Sylvain Meuris14, Panagiotis Gavrilil13, Anne-Marie Vanbellinghen1, Josiane Delogne-Desnoeck1, Claude Robyn13 and Philippe Lebrun2

1Research Laboratory on Reproduction and 2Laboratory of Pharmacology, Free University of Brussels (ULB), CP 626, 808 Route de Lennik, B-1070 Brussels and 3Department of Obstetrics and Gynecology, Hôpital Ambroise Paré, Centre Inter Universitaire, 2 Boulevard Kennedy, B-7000 Mons, Belgium
4To whom correspondence should be addressed

The purpose of this study was to evaluate, in vivo and in vitro, the influence of ritodrine and oxytocin on the placental release of human chorionic gonadotrophin (HCG) and placental lactogen (HPL). The in-vivo study was performed on maternal sera collected before and 1 h after the onset of either ritodrine treatment (50 µg i.v./min; administered to 15 women at risk of premature labour) or oxytocin infusion (2 µU i.v./min; administered to 21 women for acceleration of slow labour). The in-vitro study was performed on human term placental explants incubated in the presence of 4-400 ng ritodrine/ml or 15-1500 µU oxytocin/ml. HCG and HPL were measured by radioimmunoassay on maternal sera and incubation media. Maternal circulating concentrations of HCG and HPL remained unaffected after 1 h of ritodrine or oxytocin treatment. The in-vitro release of HCG and HPL by placental explants was not modified when ritodrine or oxytocin was added to the incubation media. The lack of influence of ritodrine and oxytocin on the placental secretion of HCG and HPL suggests that β2-adrenergic and oxytocin receptors are not involved in the releasing process. Key words: β-adrenergic/chorionic gonadotrophin/oxytocin/placenta/placental lactogen

Introduction

Ritodrine is a potent β2-adrenergic agent which is widely used in obstetrics to suppress uterine contractions in the management of pre-term labour (Wesselius-de Casparis et al., 1971). Conversely, oxytocin is an endogenous nonapeptide currently used as a uterotonic agent to increase the force, frequency and duration of uterine contractions during parturition (Fuchs and Fuchs, 1984; Jenkins and Nussey, 1991). The pharmacological effects of ritodrine and oxytocin on the uterine smooth muscle result from binding to specific transmembrane G-protein coupled receptors. This in turn activates a cascade of intracellular events ultimately leading to variations in cytosolic calcium concentration. Their opposing effects on the myometrium, causing relaxation or contraction, appear, in the case of ritodrine, to be the consequence of a decrease in cytosolic calcium concentration or, in the case of oxytocin, a mobilization of the intracellular calcium pool (for a review, see Wray, 1993).

Modifications of the cytosolic free calcium concentration are also known to elicit variations in the secretory pattern of numerous endocrine tissues, including placenta. Indeed, the placental secretion of human chorionic gonadotropin (HCG) and/or placental lactogen (HPL) can be stimulated in vitro by an increase in cytosolic calcium (Hussa, 1977; Welsch, 1979; Béliele et al., 1989; Polliotti et al., 1990, 1992; Meuris et al., 1994). The effects of ritodrine and oxytocin on these placental hormone secretions remain, however, controversial. Spellacy et al. (1978) and Ylikorkala et al. (1978) failed to demonstrate any modulating effect of ritodrine infusion on the circulating concentrations of HCG and HPL. By contrast, Schreyer et al. (1989) found this drug to increase circulating concentrations of HPL, and it also increased HCG release by cultured trophoblastic cells (Oike et al., 1990). Lastly, another report suggests that oxytocin might modify HCG pulsatile secretion by placental explants (Tal et al., 1991).

Since ritodrine and oxytocin have been shown to elicit opposing effects on the cytosolic calcium concentration, we decided to examine further the effect of pharmacological doses of both drugs on the placental secretion of HCG and HPL. In-vivo and in-vitro experiments were conducted in order to characterize a putative effect of these two drugs.

Materials and methods

The protocol of this investigation was approved by the Ethical Committee of the Faculty of Medicine of the Free University of Brussels (ULB), Belgium.

In-vivo study

The first group of patients, hereafter referred to as the ritodrine group, consisted of 15 pregnant women who were at risk of premature labour. Uterine contractions had been detected by cardiotocography prior to treatment. The mean age of the patients and the mean gestational age of their fetuses were 27.7 ± 1.7 years and 35.8 ± 1.1 weeks respectively. Ritodrine (Pre-Par, Solvay, Brussels, Belgium) was administered i.v. (100 mg/l) at a starting rate of 50 µg/min. In 14 out of 15 patients, this initial infusion rate was considered sufficient to control uterine contractions.

The second group of patients, hereafter referred to as the oxytocin group, consisted of 21 pregnant women. The mean age of the patients and the mean gestational age of their fetuses were 24.4 ± 0.9 years and 39.4 ± 0.2 weeks respectively. Treatment was initiated for
acceleration of slow labour, as shown by partogram. Oxytocin (Syntocinon; Sandoz, Basle, Switzerland) was administered i.v. (10 UI) at an initial rate of 2 mU/min. The rate of infusion was increased in 17 out of 21 women after 1 h of treatment.

Blood samples were collected, after informed consent was obtained, from all 36 women immediately before administration of ritodrine or oxytocin and again 1 h after infusion. After centrifugation, serum samples were stored at -20°C until assayed.

Serum HCG and HPL were measured in duplicate in a single radioimmunoassay for all samples. Homologous HCG and HPL radioimmunoassays were performed as previously described (Polliotti et al., 1990). Their sensitivities were 1.5 mIU HCG/ml (2nd International Standard distributed by the World Health Organization) and 0.6 μg HPL/ml. The intra-assay coefficients of variation were <8% for HCG and <6% for HPL.

The statistical significance for variation in HCG and HPL concentrations after the onset of therapy was estimated using a paired Student's t-test.

**In-vitro study**

Placentas were obtained after vaginal term delivery (37–41 weeks gestation) and transferred within minutes to the laboratory. Several cotyledons were excised and basal and chorionic plates from the maternal and fetal sides removed. Fragments of trophoblast tissue were rinsed in a cold incubation medium and minced into 25 mm³ explants.

The incubation medium consisted of a HEPES-buffered solution containing (in mM) 139 NaCl, 5 KCl, 1 CaCl₂, 1 MgCl₂, 4.2 glucose and 10 HEPES-NaOH (pH 7.4). The media were supplemented with 0.1% (w/v) dialysed albumin (fraction V; Sigma Chemical, St Louis, MO, USA) and gassed with 100% O₂ to avoid bicarbonate formation. The medium contained, as required, ritodrine (Sigma) or oxytocin (UCB, Brussels, Belgium).

Explants were pre-incubated for 90 min in a shaking bath thermostabilized at 37°C. After this equilibration period, the placental explants were incubated for an additional 90 min in a HEPES-buffered physiological salt solution to which ⁴⁵Ca (20 μCi/ml; NEN Products, Brussels, Belgium) in the form of calcium chloride in aqueous solution was added. The explants were then washed for an additional 50 min period with a non-radioactive medium in order to remove extracellular ⁴⁵Ca (Lebrun et al., 1995). At 2 min intervals, groups of two placental explants were transferred through a series of 30 glass vials containing 1 ml of aerated buffer at 37°C. Collected media were assayed for HCG and HPL, as described above. In addition, they were counted for ⁴⁵Ca effluent radioactivity (Lebrun et al., 1995).

All results were expressed as the mean (±SE) together with the number of individual experiments. Each experiment was performed with at least two different placentas. The placenta-related variations in HCG and HPL release led us to express the secretory rate with reference to a baseline value (100%) defined as the amount of hormone released by the explants during the 18 min before the stimulatory period. The magnitude of the variations in hormonal release was estimated in each individual experiment from the integrated hormonal output observed during drug administration (minutes 19–42) after correction for basal value (minutes 1–18).

The statistical significance of differences between mean data was assessed using Student's t-test.

**Results**

**In-vivo study**

The mean HCG and HPL concentrations measured in the initial blood sample from the ritodrine group (15 women) were 14013 ± 2120 mIU/ml and 5.02 ± 0.49 μg/ml respectively. After 1 h of ritodrine treatment, the mean percentages of variation were -1.8 ± 2.2% for HCG and 2.3 ± 1.7% for HPL (Figure 1). These variations around the initial value were not significant.

In the oxytocin group (21 women), the initial hormonal concentrations were 11 738 ± 1950 mIU HCG/ml and 5.88 ± 0.31 μg/ml. The mean percentages of variation around the initial value averaged, after 1 h of oxytocin treatment, -1.2 ± 4.1% for HCG and 0.9 ± 1.3% for HPL. (Figure 1). These variations were not significant.

**In-vitro study**

Exposure of human placental explants to ritodrine at doses ranging from 4 to 400 ng/ml failed to provoke any significant change in HCG and HPL release (Figure 2 and Table I). In addition, exposure of explants to doses of 15, 150 and 1500 μU/ml oxytocin did not elicit any significant modification in HCG and HPL release (Figure 2 and Table I). Lastly, ⁴⁵Ca efflux measured during the same experiments was found to be unaffected when explants were treated with ritodrine or oxytocin (data not shown).

**Discussion**

Neither ritodrine nor oxytocin, used at pharmacological concentrations, was found to affect maternal circulating amounts of HCG and HPL or to modify the basal release of HCG and HPL by human term placental explants. These data as well as the absence of modifications in ⁴⁵Ca outflow, i.e. indirect measurement of transmembrane Ca²⁺ fluxes (Lebrun et al., 1995), after addition of ritodrine and oxytocin suggest the absence of functional receiving systems for these drugs in the human syncytiotrophoblastic cell.

Our results confirm the initial studies of Spellacy et al.
was observed only with ritodrine concentrations 2*10^{-8} \text{M} or et al., 1990). This HCG response has also been reported (Oike in-vitro experiments. A direct effect of the \( \alpha \)agonist ritodrine concentrations of HCG and HPL was further supported by our on HCG release by isolated first-trimester trophoblast cells therefore may mask moderate changes in HCG concentration. HCG from maternal blood lasts hours (Nisula et al., 1989) and the in-vivo studies since the half-life for disappearance of the influence of the tocolytic agent (Brettes 1976; Schreyer et al., 1989) have described an increase in HPL during at least 6 h of ritodrine treatment. More recently, Schreyer et al., 1988). Whatever the case, our modifications in the circulating concentrations of HCG and/or effects of pharmacological doses of ritodrine and oxytocin on placental secretion of HCG and HPL. The absence of an effect of ritodrine on the placental release of HCG or HPL as well as on the \( ^{45}\text{Ca} \) efflux suggests that \( \beta \)-adrenoreceptors are not involved in the releasing process of these hormones.

Incidentally, the human placenta is a non-innervated organ but a very rich source of \( \beta \)-adrenergic receptors. Placenta can therefore be viewed as a target organ for \( \beta \)-adrenergic agents which are reported to stimulate oestadiol and progesterone production by placental cells (Caritis and Zeleznik, 1980; Barnea et al., 1989). The exact localization of these receptors remains unclear. They might equip the fetal vasculature rather than the syncytiot (Schocken et al., 1980; Whitsett et al., 1980, 1982; Falkay et al., 1994). Thus, the absence of functional \( \beta \)-adrenoreceptors on syncytiotrophoblastic cells might explain the lack of a direct effect of ritodrine on both \( ^{45}\text{Ca} \) efflux and placental secretion of HCG and HPL.

The lack of an effect of oxytocin on either \( ^{45}\text{Ca} \) efflux or placental release of HCG and HPL can also be viewed, as for ritodrine, as the consequence of the absence of functional specific binding sites in syncytiotrophoblastic cells. Oxytocin receptors are known to increase drastically in the myometrium and decidua as term approaches and particularly during parturition (Fuchs and Fuchs, 1984; Jenkins and Nussey, 1991). However, no oxytocin receptor mRNA and protein have been found in human trophoblast cells, whereas they are abundant in decidua (Takeamura et al., 1994). Nevertheless, placenta seems to be a target organ for oxytocin because this hormone stimulates pro-opiomelanocortin secretion by human placental fragments (Margioris et al., 1988). Whatever the case, our results further support the concept that, despite concentrations exceeding by 10- to 100-fold those normally circulating (Leake et al., 1981), oxytocin fails to affect HCG or HPL release.

Lastly, the present study confirms the absence of deleterious effects of pharmacological doses of ritodrine and oxytocin on placental secretion of HCG and HPL.

### Table 1. Concentration-dependent effect of ritodrine and oxytocin on the release of human chorionic gonadotrophin (HCG) and human placental lactogen (HPL) by human placental explants.

<table>
<thead>
<tr>
<th>Drug added</th>
<th>Expression of HCG in time period (min):</th>
<th>Expression of HPL in time period (min):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-18</td>
<td>19-42</td>
</tr>
<tr>
<td>Ritodrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 ng/ml</td>
<td>100 ± 2.33</td>
<td>105.87 ± 4.59</td>
</tr>
<tr>
<td>40 ng/ml</td>
<td>100 ± 1.79</td>
<td>105.05 ± 4.40</td>
</tr>
<tr>
<td>400 ng/ml</td>
<td>100 ± 2.52</td>
<td>100.87 ± 2.62</td>
</tr>
<tr>
<td>Oxytocin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 ( \mu \text{M} )</td>
<td>100 ± 2.18</td>
<td>104.71 ± 2.72</td>
</tr>
<tr>
<td>150 ( \mu \text{M} )</td>
<td>100 ± 3.02</td>
<td>96.68 ± 5.23</td>
</tr>
<tr>
<td>1500 ( \mu \text{M} )</td>
<td>100 ± 2.38</td>
<td>95.26 ± 2.94</td>
</tr>
</tbody>
</table>

Figure 2. Effect of ritodrine (40 ng/ml; left panels) and oxytocin (150 \( \mu \text{M} \); right panels) on the release of human chorionic gonadotrophin (HCG) and human placental lactogen (HPL) from human placental explants. Mean values ± SE refer to four individual experiments performed with explants from two placentas.

(1978) and Ylikorkala et al. (1978) showing the absence of modifications in the circulating concentrations of HCG and/or HPL during at least 6 h of ritodrine treatment. More recently, however, Schreyer et al. (1989) have described an increase in serum HPL concentration after 3 h of ritodrine infusion in third trimester pregnancies. This increase was proposed to result mainly from an enhanced placental blood flow under the influence of the tocolytic agent (Brettes et al., 1976; Schreyer et al., 1989). A putative effect of ritodrine on circulating concentrations of HCG could not be excluded from the in-vivo studies since the half-life for disappearance of HCG from maternal blood lasts hours (Nisula et al., 1989) and therefore may mask moderate changes in HCG concentration.

The lack of an in-vivo effect of ritodrine on circulating concentrations of HCG and HPL was further supported by our in-vitro experiments. A direct effect of the \( \beta \)-agonist ritodrine on HCG release by isolated first-trimester trophoblast cells has also been reported (Oike et al., 1990). This HCG response was observed only with ritodrine concentrations \( \geq 10 \mu \text{M} \) or \( \geq 3.2 \mu \text{g/ml} \). Such a threshold can be viewed as exceeding by 10- to 100-fold the ritodrine plasma concentrations effective for inhibiting uterine contractions (Caritis et al., 1985; Caritis, 1988). All these data suggest that the circulating concentrations of ritodrine usually reached in order to prevent uterine contractions are without any significant effect on the placental release of HCG and HPL. The absence of an effect of ritodrine on the placental release of HCG or HPL as well as on the \( ^{45}\text{Ca} \) efflux suggests that \( \beta \)-adrenoreceptors are not involved in the releasing process of these hormones.
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