Author's response to reviews

Title: Inhibition of the oxytocin receptors delays gastric emptying

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Author's response to reviews: see over
Answers to the reviewers and Editor Malmö 31 jan 2006

Thank you very much for the offer to resubmit a revised version of the manuscript. We have now carefully considered all issues raised by the referees and accordingly revised the manuscript herewith submitted. Our actions taken to the raised points are found on the following pages. We have also changed the title.

Sincerely, Bodil Ohlsson

Reviewer nr 1
1. The ultrasound method used in the present study is a simplified and standardized reproducible, well-tolerated and clinically applicable method for the assessment of gastric emptying rate (Darwiche et al. 1999). It has been shown that the use of this standardized real-time ultrasonography to determine gastric antral cross-sectional area in a single section of the stomach is a valid method for estimating gastric emptying rate giving highly reproducible results regarding interobserver and intraobserver measurement errors. The gastric emptying rate was expressed as percent reduction in antral cross-sectional area from 15 to 90 minutes after meal ingestion in our present study since previous ultrasonographic measurements has showed a postprandial maximal antral area at 15 minutes, continuously decreasing with time, and reaching a plateau 45 to 90 minutes after the end of the ingestion of the standardized semisolid breakfast meal (300 g rice pudding, 330 kcal) (Darwiche et al 1999, Darwiche et al 2003). We have in these earlier studies seen that further measurements every 5-10 min do not render more information than GER between 15-90 min. Scintigraphic measurements of total stomach emptying (the gold standard method for the measurements of gastric emptying) has also been compared with ultrasonographic measurements of changes in antral area as estimates of antral emptying, showing concordant results between 15 and 90 minutes regarding the residual radioactivity and antral ultrasonographically measured distension curves (Fig 1)(Darwiche et al 2003 ). A strong significant correlation has also been shown between the ultrasonographic gastric emptying rate and scintigraphic half-time values ($r = -0.94; P < 0.001$). This last reference is now included in the method section (page 5, last paragraph). After ingestion or intragastric administration of equal amounts of liquid, antral volumes determined by ultrasonography show a wide intersubject variability (Ricci et al 1993). Therefore, it should be more important to evaluate changes in the antral area over time, as a measure of GER, instead of comparing the antral area at a fixed time between subjects. This is clarified on page 5, last paragraph.

2. We chose to measure the antral area at 2 different occasions by reasons mentioned above. As we correlate the satiety scores to the antral area, we measured these scores at the same time.

3. You are right that dose-response curves could have been performed. However, we have earlier studied the effect of intravenous oxytocin on colonic peristalsis (Ohlsson et al 2004). We then used 2 dosages of oxytocin: 20mU/min and 40 mU/min. In some cases, 2 experiments in the same person were performed with the 2 different doses, and in some cases one dose in some persons and the other dose to other persons. We could in that study not discover any difference between the 2 doses used, explained in the methods (page 5, second paragraph). Both doses evoked increased peristalsis in the colon. As 40mU/min of oxytocin is the highest dose recommended by the drug company (Novartis ©), and we have used this dose
earlier, we chose that dose also in the present experiment. Further, this dose is commonly used and established in the obstetrics to evoke and sustain uterine contractions (Weis et al 1975, Parfitt et al 1999, Carvalho et al 2004, Vimala 2005). Hashmoni et al (1979) used the same dosage when treating postvagotomy gastric atony. Thus, it is a well established dose for many purposes. Nevertheless, after the finding in the present study, a new dose-response curve specifically for gastric emptying would be to prefer, as mentioned in the discussion (page 10, last paragraph).

Five persons had their gastric emptying increased and 5 had it decreased by oxytocin. Thus, we can conclude that this dose of oxytocin does not affect gastric emptying significantly. The dose of Petring (1989) was 10 times higher. This may have affected vasopressin receptors instead of oxytocin receptors. This was our first study in this field. We will go on with further examinations on the presence of vasopressin response to a meal and if there are vasopressin receptors in the human gastrointestinal tract. Maybe it is vasopressin that is most important.

For discussion of the method, see answer question nr 1.

4. We have earlier measured oxytocin levels in response to a similar meal twice. The oxytocin levels rose from 1.0±0.17 basally to 1.3 ±0.26 pmol/l postprandially, p=0.02 (Ohlsson et al 2002) and from 208.1±148.7 basally to 250.6±166.9 pg/ml postprandially, p=0.02, respectively (Chebib et al., unpublished data). Oxytocin was measured by two different methods, explaining the difference in values. In the present study we did not collect blood samples as we have did it twice before, and the measurements are very expensive.

5. Atosiban acts by competing with oxytocin for its receptors. This results in a dose-dependent inhibition of contractility. A number of preclinical toxicological and receptor binding studies have been conducted in vitro and in vivo in animals, and in vitro in human tissue preparations. The receptor affinities of atosiban, vasopressin and oxytocin were demonstrated to be similar in myometrial membrane preparations (Ryden et al 1990, Maggi et al 1994).

In vitro, in human myometrium, atosiban was shown to completely inhibit oxytocin-induced increases in intracellular calcium but did not prevent this increase when it was either potassium- or prostaglandin E2-induced (Thornton et al 1993, Phaneuf et al 1994). Further studies on its effect on intracellular secondary messengers have shown a dose-dependent inhibition of oxytocin-stimulated inositol phosphate production (Lopez-Bernal et al 1989). These findings indicate that atosiban is specific to the action of oxytocin, and that an additional intracellular process may be attributed to atosiban following receptor binding. Another observation in rats after chronic treatment with oxytocin demonstrated a down regulation in receptor numbers with continued exposure to oxytocin, whereas chronic exposure to atosiban appeared to increase the number of receptors and the affinity for the receptors (Engström 1988). The intracellular events are stronger against oxytocin than against vasopressin (Phaneuf et al 1994). This is discussed in the discussion section (page 8-9).

6. That the antral area was smaller in the atosiban group compared to controls may be caused by a retention of the meal in the proximal stomach. This is a speculation and You may be right, it can as well be due to an early transient emptying. Both speculations are now mentioned in the discussion (page 8, first section). However, the former explanation seems more logical than a strong initial emptying during the first 5-10 min, followed by a decrease.

7. The motility in the stomach, ileum and caecum is inhibited by oxytocin in animals (Wu et al. 1993). When performing immunohistochemistry in another study, we were not able to demonstrate oxytocin and its receptor in the rat gastrointestinal tract (Frank Sundler and Bodil
Ohlsson, unpublished data). This might explain why the effects evoked by oxytocin on gastric and intestinal motility in rat are mediated by release of cholecystokinin (CCK) and CCK receptors, and differ from oxytocin effects evoked in human (Wu et al. 1993, Liu et al 2002). Thus, the effect of oxytocin on rat gastrointestinal tract is indirect, and CCK is known to inhibit gastric emptying, as now discussed in the discussion section (page 10, line 14).

8. We have added a new figure, fig 2, where the individual GER are shown during saline and atosiban administration. As described above under point 1, it is not convincing to scatter the antral area as there are great interindividual variability.

Reviewer 2
1. Please, see above under rev 1 question 1 and 8.
2. We have earlier measured oxytocin levels in response to a similar meal twice. The oxytocin levels rose from 1.1±0.17 basally to 4.4±0.8 pmol/l postprandially, p=0.02 (Ohlsson et al 2002) and from 208.1±148.7 basally to 250.6±166.9 pg/ml postprandially, p=0.02, respectively (Chebib et al., unpublished data). Oxytocin was measured by two different methods, explaining the difference in values. The values for healthy controls are published in Ohlsson 2002, and the other values are written in a manuscript prepared for submission during this spring. We did not collect blood samples in the present study as we have measured oxytocin levels twice earlier, and it is a very expensive analyse. We do not find it necessary to include these values in the discussion.

3. We did not collect blood samples or measure the oxytocin levels in the present study. An earlier publication has shown that a similar dose of oxytocin (1 pmol/kg/min) in man rose the oxytocin levels in plasma from 2-3 pg/ml to around 50 pg/ml. In the same study, the oxytocin levels were stable in the control group receiving saline during the experiment (Rasmussen et al 2004). This is a 25-fold increase, and much greater than the postprandial oxytocin increase described in question 2. This ref is now included in the methods (page 5, second paragraph).

4. Atosiban acts by competing with oxytocin for its receptors. This results in a dose-dependent inhibition of contractility. A number of preclinical toxicological and receptor binding studies have been conducted in vitro and in vivo in animals, and in vitro in human tissue preparations. The receptor affinities of atosiban, vasopressin and oxytocin were demonstrated to be similar in myometrial membrane preparations (Ryden et al 1990, Maggi et al 1994). In vitro, in human myometrium, atosiban was shown to completely inhibit oxytocin-induced increases in intracellular calcium but did not prevent this increase when it was either potassium-or prostaglandin E2-induced (Thornton et al 1993, Phaneuf et al 1994). This intracellular effect was stronger against oxytocin than vasopressin (Phaneuf et al 1994). Further studies on its effect on intracellular secondary messengers have shown a dose-dependent inhibition of oxytocin-stimulated inositol phosphate production (Lopez-Bernal et al 1989). These findings indicate that atosiban is specific to the action of oxytocin, and that an additional intracellular process may be attributed to atosiban following receptor binding. Another observation in rats after chronic treatment with oxytocin demonstrated a down regulation in receptor numbers with continued exposure to oxytocin, whereas chronic exposure to atosiban appeared to increase the number of receptors and the affinity for the receptors (Engström 1988). This is discussed in the discussion section (page 8-9).

References


Vimala N, Mittal S, Kumar S. Sublingual misoprostol versus oxytocin infusion to reduce blood loss at cesarean section. Int J Gyn Obs 2005;in press


Figure 1. The correlation between scintigraphy and ultrasonography from Darwiche et al 2003