Effect of oxytocin on duodenal motility in female rats
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Introduction
Oxytocin is a well-acknowledged nonapeptide hormone produced in the paraventricular and supraoptic nuclei of the hypothalamus, classically known to facilitate parturition and lactation. Actions of oxytocin commence right before pregnancy, continues during and after birth, and travels from the brain to the heart and throughout the entire body, triggering or modulating a full range of physiological functions and emotions.

In the last decade, additional evidence has indicated that oxytocin may play a role in the regulation of gastrointestinal functions such as immune response to inflammation [1], sensations [2], and motility [3]. Transcripts encoding oxytocin and those encoding oxytocin receptors (OTRs) have been reported to be present in most segments of the gut in adult humans [4] and rats [5] and on the neurons of the enteric nervous system of guinea pigs [6].

With regard to the effect of oxytocin on gastrointestinal motility in humans, one study reported no significant effect or advantage over placebo in terms of the effect of oxytocin on constipation in women [7]. Other studies showed that oxytocin accelerates and oxytocin antagonists delay gastric emptying in humans [8,9]. A more recent study showed that gastroparesis is associated with oxytocin deficiency [10].

The reports describing the effects of oxytocin on gastrointestinal motility in animals were contradictory. Earlier studies showed inhibitory effects of oxytocin on the motility of the proximal colon in rabbits [11]. Also, administration of oxytocin to the dorsal motor nucleus of the vagus was found to inhibit gastric motility in anesthetized rats [12] and non-stressed mice [13]. Moreover, studies carried out by Wu et al. [14,15] and Liu et al. [16] found that gastric emptying and gastrointestinal transit were inhibited by oxytocin in female and male rats.

In contrast, some recent studies have reported stimulatory effects of oxytocin on gastrointestinal motility in animals. Systemic oxytocin dose dependently increased the contraction of muscle strips of the gastric body and antrum [17] as well as duodenal motility [18] in rabbits. The excitatory effect of oxytocin was also reported in...
distal colonic smooth muscle strips of control and antenatal maternal hypoxia mice [19].

The aim of the present study was to investigate the effect of oxytocin on duodenal motility in normal female rats both in vivo and in vitro and to discuss the controversial results in animal studies.

Materials and methods

Animals
In the present study, a total of 30 adult female Wistar albino rats, weighing 200–250 g, were used. Rats were purchased from the Egyptian Organization for Biological Products and Vaccines (VACSERA) (El-Agouza, Cairo) and maintained in the animal house of the Physiology Department under standard conditions of boarding and were given a regular diet of bread, vegetables, and milk with free access to water.

Experimental protocol
The rats used in this study underwent an in-vivo and in-vitro study.

In-vivo study
Rats subjected to the in-vivo study were classified into the following groups:

Group I: This group comprised control rats (n = 10) that received no treatment but were kept in the same environmental conditions as the other groups.

Group II: This group comprised oxytocin-treated rats (n = 10) that received daily intraperitoneal injections of oxytocin (Sigma, Chemical Co., St. Louis, Missouri, USA) at a dose of 5 μg/kg body weight [18] for 7 days. This dose was the lowest used by many investigators and was considered a low pharmacological dose [11–13].

In-vitro study
Group III: This group comprised in vitro oxytocin subjected rats (n = 10). To examine the in-vitro effect of oxytocin on duodenal motility, different doses of oxytocin (0.022, 0.11, 0.22, and 1.1 μg/30 ml bath) were added to the organ bath. The effect of oxytocin at a dose of 0.022 μg/30 ml bath was recorded before and after full atropinization (1.5 mg/30 ml bath).

Experimental procedures
On the day of the experiment, rats fasted overnight, except for free access to water, were weighed and anesthetized by intraperitoneal injection of thiopental sodium (EIPICO, 10th of Ramadan City, Egypt) at a dose of 40 mg/kg. A midline abdominal incision was made, abdominal muscles and viscera were separated, and different parts of the gastrointestinal tract were identified and segments of duodenum dissected and rapidly immersed in Tyrode’s solution [20].

Intestinal motility studies
Preparation of the intestine and recording of in-vitro intestinal motility were performed according to the technique described by Mohamed et al. [20]. Segments of duodenum about 1 cm long were suspended in an organ tissue bath containing 30 ml of warmed (37°C) Tyrode’s solution continuously bubbled with 95% O2 and 5% CO2. The examined duodenal segment was fixed at one end in the bath, and the other end was connected to an isometric force displacement transducer (Biegestab K30; Hugo Sachs Elektronik, March-Hugstetten, Germany) for recording of isometric contractions. The duodenal segment was left in the organ bath for a stabilization period of 20 min before recording. Intestinal motility was recorded on a two-channel oscillograph (Washington MD2; Bioscience, Seattle, Washington, USA) in which the downstroke represents contraction and upstroke represents relaxation. The sensitivity of recording was 0.1. The speed of recording was 2.5 mm/s.

Calculation of the results
From a calibration curve, the amplitude of the recorded contractions measured in millimeters and the equivalent force of contraction in grams were obtained. The duration of each contraction was calculated by measuring the distance of contraction in mm and dividing it by the speed of recording; the result was expressed in seconds.

Intestinal motility parameters
The following parameters were used to evaluate the intestinal contractions [21]:

1. Frequency of contraction (number of contractions/min): The average number of contractions/min was counted within the recording time.
2. Average duration of contraction (s): The average duration was calculated by dividing the sum of the duration of contractions in seconds within the recording time (1 min) by the number of contractions.
3. Average force of contraction (g): The average force was determined by dividing the sum of contraction forces in grams within the recording time by the number of contractions.
4. Motility index (g min): The motility index was obtained from summation of the product of the contractile force in grams and the duration of each contraction in 1 min [22,23].

Statistical analysis
Data were expressed as mean ± SD. The Student’s t-test for paired and unpaired data was performed to assess the statistically significant intragroup and intergroup differences, respectively. All statistical data and statistical significance, were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, Illinois, USA), version 16. A P value less than 0.05 was considered statistically significant.

Results

In-vivo study
Table 1 and Fig. 1 show that the oxytocin-treated group exhibited a significant increase in the average force of
duodenal contraction \( (P<0.01) \) and motility index \( (P<0.01) \) but a significant decrease in the duodenal frequency of contraction \( (P<0.05) \) when compared with the control group. However, the changes in the average duration of contraction were insignificant.

**In-vitro study**

**Responses to low doses of oxytocin**

As shown in Table 2 and Fig. 2, in-vitro administration of oxytocin at a dose of 0.022 \( \mu \)g/bath resulted in a significant increase in the duodenal average force of contraction \( (P<0.05) \) and motility index \( (P<0.05) \) when compared with their respective baseline values. However, nonsignificant results were observed in the frequency of contraction and average duration of contraction.

Similarly, oxytocin at a dose of 0.11 \( \mu \)g/bath resulted in a significant increase in the duodenal average force of contraction \( (P<0.01) \) and motility index \( (P<0.05) \) when compared with their basal values, whereas the changes in frequency of contraction and average duration of contraction did not reach statistical significance.

**Table 1 Duodenal motility parameters of control and in-vivo oxytocin-treated groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Oxytocin-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of contraction (W/min)</td>
<td>38.7 ± 3.65</td>
<td>35.5 ± 2.32*</td>
</tr>
<tr>
<td>Average duration of contraction (s)</td>
<td>1.50 ± 0.11</td>
<td>1.56 ± 0.14</td>
</tr>
<tr>
<td>Average force of contraction (g)</td>
<td>0.45 ± 0.14</td>
<td>0.76 ± 0.22*</td>
</tr>
<tr>
<td>Motility index (g/min)</td>
<td>26.1 ± 8.18</td>
<td>42.6 ± 12.8*</td>
</tr>
</tbody>
</table>

Data are means ± SD. The number of rats per group is 10.

*Significant difference from the control group, calculated by the Student’s \( t \)-test for unpaired data at \( P<0.05 \).

**Discussion**

The results of the present work on the effect of oxytocin injected intraperitoneally for 7 days showed that duodenal motility significantly increased with respect to the average force of contraction and the motility index. These results are in accordance with those of both human and animal studies [19,24]. The motility alterations were not affected by atropine, indicating that oxytocin does not act through cholinergic muscarinic receptors. In a previous study, the stimulatory effect of oxytocin on gastric motility was not found to be influenced by hexamethonium but was blocked by oxytocin antagonist (atosiban), suggesting that this is a direct effect on OTRs expressed on circular and longitudinal muscle of the stomach in rats [18,19].

The prokinetic effect of oxytocin on the gut has been assumed to be similar to its effect in uterine myometrium and mammary myoepithelial cells – that is, intracellular release of \( \text{Ca}^{2+} \), which leads to muscle contraction by activation of myosin light-chain kinase [25]. The intracellular signaling pathways are activated by Gq protein besides the activation of phospholipase C, which controls the generation of inositol trisphosphate and diacylglycerol.
which in turn causes liberation of Ca$^{2+}$ from the intracellular stores, opening of plasmalemmal Ca$^{2+}$ channels, and activation of protein kinase C [26,27]. Moreover, OTR activation leads to stimulation of phospholipase A2 production and an increase in cyclooxygenase 2 levels, both resulting in increased production of prostaglandins [28].

As the rats used in our study were females in their reproductive period, the estrogen hormone may have a role in the response to oxytocin, as the excitatory effect of oxytocin on colon motility in ovariecctomized rats was shown only after estrogen replacement, indicating that estrogen upregulates OTR on the rat duodenum [24].

The excitatory effect of oxytocin on intestinal motility could also be attributed to its effect on the oxidative status. High levels of malondialdehyde, indicating lipid peroxidation, and low levels of glutathione, a key antioxidant, may cause decreased intestinal motility [28] and oxytocin was found to decrease malondialdehyde and increase glutathione levels [29,30].

The current findings of the in-vitro study showed that at low doses of oxytocin there was stimulation of duodenal motility, whereas a high dose of oxytocin induced an inhibitory effect on motility. The inhibitory effect of oxytocin on intestinal motility reported previously in rats [12–16], which appears to be contradictory to the results of our in-vivo study, was seen in our in-vitro studies when higher doses of oxytocin were used. The inhibitory effect was in accordance with that seen in other reports [14,31]. The inhibitory effect appears to be related to the very high dose of oxytocin used in these studies (0.2–0.8 mg/kg) and $10^{-5}$–$10^{-4}$ mol/l (0.10–10 mg range) and the same dose in a very recent study $10^{-5}$–$10^{-7}$ M (1–10 mg) [32] whereas in our in vivo study we used 5 ug/kg.

It is a well-known phenomenon that stimulation of a receptor by increasing dosage may result in a bell-shaped response [17,33]. The divergent effect of the different dosages could be explained on the basis of the diversity of OTR signaling and the coupling of OTR to different G proteins, as $G_o$, $G_1$, and $G_i$ exhibiting opposite effects [26] suggest an explanation for the dual in-vitro oxytocin responses.

Furthermore, the inhibitory effect of high levels of oxytocin on intestinal motility was explained by the high level of cholecystokinin (CCK) [34]. Moreover, the investigators in this study used a very high dose of oxytocin (0.2–0.8 mg/kg). This level of CCK was not affected in rats injected with the low dose of oxytocin.
comparable to that of our study, and the excitatory effect was not abolished by the CCK receptor antagonist (devazepide) [18]. The inhibitory effects of high oxytocin doses may also be attributed to the effect of oxytocin on another β-2 adrenoceptors coexpressed with OTRs on the duodenum and OTR coexpression may be coupled to a novel β-2 adrenoceptor pathway which produces this inhibitory effect [35].

From the present data, we conclude that low doses of oxytocin increased duodenal motility in female rats possibly through a direct mechanism and that higher levels of oxytocin may have an inhibitory effect, which could be explained by an indirect mechanism.

Acknowledgements
Conflicts of interest

There are no conflicts of interest.

References


