

Short communication

Oxytocin is elevated in plasma of 10-day-old rats following gastric distension

Eric E. Nelson^{a,*}, Jeffrey R. Alberts^a, Ying Tian^b, Joseph G. Verbalis^b

^a Department of Psychology, Indiana University, Bloomington, IN, USA

^b Division of Endocrinology, Georgetown University School of Medicine, Washington, DC, USA

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Abstract

In adult rats, oxytocin (OT) has been shown to reduce the intake of both food and fluids, and oxytocinergic cells are activated by gastric distension and administration of the intestinal peptide cholecystokinin (CCK-8). These and other findings indicate that OT can play a role in inhibiting ingestion under some conditions. A previous study has shown, however, that oxytocinergic cells are unresponsive to CCK-8 in 2-day-old rats. We report here that OT is elevated in the plasma of 10-day-old rats after induction of gastric distension with both mother's milk and saline. These results indicate that the vagal–hypothalamic axis becomes mature between 2- and 10-days of age in infant rats. © 1998 Elsevier Science B.V. All rights reserved.

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Several lines of evidence have indicated that the neurohormone oxytocin (OT) plays an important role in inhibiting ingestion in adult rats [19]. For example, intracerebroventricular administration of OT reduces food intake [1,10]; and a number of other manipulations which are known to reduce food intake such as gastric distension, administration of the intestinal peptide cholecystokinin-8 (CCK-8), and exposure to illness producing agents such as lithium chloride result in activation of OT neurons, and elevations of OT in plasma [13,20,21]; and finally, i.c.v. administration of an OT antagonist has been shown to attenuate the effects of various anorectic treatments [11].

Although many of the factors which act to regulate ingestion in adult rats are not functional in neonates [2–4], both CCK-8 and gastric distension have been shown to inhibit milk intake in rats as young as 6-days of age [4–6,8,9,12,16]. These findings would suggest that OT cells are also likely to be functional and responsive to digestive signals relatively early in development. Indeed, immunohistochemical and electrophysiological studies have demonstrated that OT neurons are both present and functional even prior to birth in rats [18]. However, re-

cently it was demonstrated that oxytocinergic cells do not respond to systemic administration of CCK-8 in 2-day-old rats [15]. This unresponsiveness of neonatal OT neurons is thought to be the result of immaturity in the catecholaminergic interneurons which project from the nucleus of the solitary tract (the medullary nucleus to which vagal afferents project) to the oxytocinergic cells in the hypothalamus [14]. The ability of gastric distension and CCK-8 to inhibit ingestion in 6-day-old pups, however, suggests that this pathway may be mature by the end of the first week of life.

In the present study, we investigated the ability of gastric distension to induce activation of oxytocinergic cells in rats during the second week of life. Although the parvocellular OT neurons appear to be primarily responsible for the inhibition of ingestion, previous studies have shown that both parvocellular and magnocellular OT neurons are activated simultaneously by several anorectic treatments, and consequently for these types of treatments plasma OT levels can serve as a marker for parvocellular OT activity as well [19]. Therefore, in the present study, we measured plasma OT levels following intragastric infusion of both isotonic saline and mothers' milk in 10-day-old rats. In addition, because a previous study has reported the presence of OT in human breastmilk [7], we also measured OT content in the milk of lactating rats to determine whether milk may serve as a vehicle for delivery of

* Corresponding author. Present address: Harlowe Primate Lab, 22 N. Charter, Madison, WI 53715, USA.

maternally produced OT to suckling infants, and thereby potentiate or substitute for endogenous OT production.

Subjects: All experiments were conducted on 10-day-old male and female Sprague–Dawley rat pups. Pups were born in the laboratory animal colony, and maternity cages were checked daily for births. The day of discovery was considered day 0. Pups were housed with the dams in 45 (l) × 26 (w) × 20 (d) cm plastic tubs lined with wood chip bedding. Litters were culled to eight at 3-days of age. The colony was on a 12:12 LD cycle, with lights on at 0800 h. Food and water were available to the dam ad libitum.

Obtaining milk: All pups were removed from the mother approximately 16 h prior to milking to increase the availability of milk in the dam. Dams were then lightly anesthetized with Aerrane inhalation (3% isoflurane in oxygen) for a 3–4-h period and milked by hand. In order to facilitate milk ejections, four of the 16 h deprived pups were allowed to suckle on the anesthetized dam during milking. Milk was manually extracted from the unsuckled nipples by applying light pressure to the nipple and surrounding tissue with forceps while pups were suckling. As milk appeared at the surface of the nipple, it was aspirated with a syringe and immediately placed in a 1.5 ml tube on dry ice. This procedure yielded roughly 2.0 ml of milk from each lactating dam in a 2- to 4-h period. At the end of the milking procedure, milk bands were clearly visible in the ventrum of all pups which were allowed to suckle, indicating that milk ejections had indeed taken place. Milk was stored in a -26°C freezer until assay was performed, or used for intragastric infusions.

Intragastric infusion: Experimental pups were removed from the mother and placed in a 32°C incubator for a 6-h period. At the end of the 6-h deprivation period, pups received intragastric infusion of milk ($n = 24$), 0.9% saline ($n = 24$), or sham treatment in which feeding tube was inserted but no infusion took place ($n = 20$). Infusions were performed by placing a feeding tube into the oesophagus, and infusing a volume of 0.5 ml (approximately 2% of bodyweight) over roughly a 15–20 s period. Thirty seconds, 5, 15, or 30 min after infusion, pups were decapitated and trunk blood was collected into 1.5 ml tubes containing 100 μl heparin. Gastric infusion was verified by examining stomachs for milk or saline content after blood was collected. Blood was immediately spun at 4000 rpm in a centrifuge at 0°C for 9 min. Plasma was separated and placed on dry ice. Plasma samples remained on dry ice until assay was performed. OT content of both milk and plasma samples was determined by radioimmunoassay after acetone–ether extraction as described previously [17].

Results: As can be seen in Fig. 1, OT levels were markedly elevated following infusion of both milk and saline. These data were analyzed with a two-factor ANOVA for infusion group and time post infusion. A significant main effect was found for both infusion, $F(1,56) = 11.92$, $p < .001$; and time, $F(3,56) = 13.22$, $p < .001$, and a significant interaction effect was also found, $F(6,56) = 4.15$,

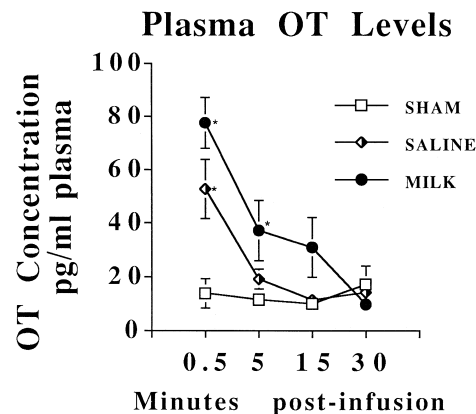


Fig. 1. OT levels detected in plasma of pups after receiving intragastric infusion of mothers' milk (black circles); 0.9% saline (black triangles); or sham infusion (open squares). Plasma levels were measured 0.5, 5, 15, and 30 min after infusion.

$p < .01$. Individual comparisons were then performed on each treatment group and the sham group at each time point with one tailed t -tests. These comparisons revealed a significant elevation from the control group for both saline $t(9) = 2.93$, $p < .05$ and milk-infused subjects $t(9) = 5.37$, $p < .01$ at the first time point, and for the milk-infused subjects at the 5-min time point, $t(9) = 2.05$, $p < .05$. OT was also found in all rat milk samples assayed, however, the levels were quite low. OT levels ranged from 0.5 to 2.9 pg/ml in the five samples assayed with a mean of 1.54 pg/ml.

Thus, we observed clear elevation of OT in the plasma of 10-day-old rats after induction of gastric distension by both mothers' milk and physiological saline. This effect was apparent immediately after infusion and persisted approximately 5 min following saline infusion and 15 min following milk infusion. A similar response, although of lesser magnitude, has been observed in adult rats after gastric balloon inflation [13] and therefore, we believe the present response is a result of gastric distension induced by both saline and milk.

The potentiated OT response to milk over saline infusions was an interesting and unexpected finding. Although OT was detected in rat milk, the difference between the milk and saline infusions was far greater than OT levels that were found in milk and therefore this difference cannot be directly attributed to OT content in the milk. We believe this difference is probably the result of constitutional differences between milk and saline (i.e., viscosity, fat content, etc.), although this awaits further study.

In summary, the present results demonstrate that the axis between vagal afferent signals and hypothalamic OT cells which has been well-characterized in adults [13] is functional by 10-days of age in rats. This finding and those of previous studies [15] indicate that the maturation of the vagal–hypothalamic axis occurs between 2- and 10-days of age in rats.

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