Oxytocin does not directly affect vascular tone in vessels from nonpregnant and pregnant rats

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Miller, M. E., S. T. Davidge, and B. F. Mitchell. Oxytocin does not directly affect vascular tone in vessels from nonpregnant and pregnant rats. Am J Physiol Heart Circ Physiol 282: H1223–H1228, 2002; 10.1152/ajpheart.00774.2001.—Recent evidence suggests oxytocin (OT) may regulate vascular tone. OT and its receptor (OTR) have been identified in the rat heart and great vessels. Expression of OT and OTR is increased in some tissues during pregnancy. We hypothesized that OT/OTR system may be a physiological regulator of vascular tone and mediate the decreased vascular resistance noted during pregnancy. Using a wire myograph system, we measured changes in vascular tone in response to OT in small mesenteric arteries, uterine arcuate arteries, and thoracic aorta from nonpregnant and pregnant rats. Additionally, we used reverse transcriptase-polymerase chain reaction (RT-PCR) to measure mRNA for OTR in these vascular tissues. Although OTR mRNA was identified by RT-PCR, OT did not elicit a vasodilatory effect in any of the vessels studied. High concentrations of OT (>10^-8 M) caused vasoconstriction that was eliminated by a specific vasopressin V1a receptor antagonist. Although it may have an indirect effect in regulation of peripheral resistance, we conclude that OT is unlikely to play a direct role in the physiological regulation of vascular tone.

Oxytocin; vasoconstriction; oxytocin receptor; vascular smooth muscle; peripheral resistance; vasopressin V1a receptor

Recent studies have demonstrated the presence of oxytocin (OT) and its receptor (OTR) in tissues from the major conduit vessels and the heart (10, 12, 19, 20, 22, 23, 38, 41, 42). OT stimulates release of atrial natriuretic peptide (19) from cardiac tissues and nitric oxide from human umbilical vein endothelial cells in culture (42). These findings have led to the suggestion that OT may be an important mediator of vascular function (20). Pregnancy is a physiological state where vasodilation is necessary to accommodate a 40–50% increase in blood volume required to meet the oxygen and nutritional requirements of the growing uterus and developing fetuses (18, 43). This is accompanied by an attenuated responsiveness of maternal vessels to pressor agents (17, 29, 30, 39, 40, 45) and an enhanced response to vasodilators (28, 31). The mechanisms underlying these vascular changes are not clearly understood.

Because pregnancy is known to induce the expression of OTR in uterine tissues (14, 15, 35–37), we hypothesized that OT acting through OTR may be an important vasodilator and may mediate the decreased vascular resistance characteristic of the pregnant state. The objective of this study was to determine and compare the direct effects of OT on vascular tone in small mesenteric and uterine arcuate vessels from nonpregnant and pregnant rats. In addition, we sought to determine whether mRNA for OTR was present in these vascular beds.

Materials and Methods

Animals. All animal protocols were in accordance with the guidelines issued by the Canadian Council on Animal Care and were accepted by the University of Alberta Animal Welfare Committee. Three-month-old female nonpregnant and late pregnant Sprague-Dawley rats were used in these protocols. Pregnant rats (usual day of delivery is day 22 of gestation) were used on day 19 of gestation. On the day of experiment, the rats were euthanized with pentobarbital sodium (50 mg/kg body wt). The small mesenteric arteries and uterine arcuate arteries were collected. These vessels were chosen because they represent the small “resistance” arteries with diameter in the range of <0.5 mm that appear to be important mediators of total peripheral vascular resistance (9). We have used mesenteric vessels previously to study pregnancy adaptations (13). Additionally, there appear to be differences in the adaptations to pregnancy between the mesenteric and uterine vascular beds (5, 12). Vascular tissues were cleanly dissected from adherent connective and adipose tissues before being used in the wire myograph system or snap-frozen for subsequent reverse transcriptase-polymerase chain reaction (RT-PCR). Aortic rings also were collected and examined for comparison to previous data in the literature.

Materials. Phenylephrine, methacholine, and OT were purchased from Sigma Aldrich Canada (Oakville, Ontario, Canada). The arginine vasopressin (AVP) V1a receptor antagonist d(CH2)6[ Tyr(Me)2,Dab3]AVP (7) was a kind gift from Dr. Maurice Manning from the Medical College of Ohio (Toledo, OH). HEPES-buffered physiological saline solution (142 mM NaCl, 4.7 mM KCl, 1.17 mM MgSO4, 1.56 mM

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CaCl₂, 1.18 mM K₂HPO₄, 10 mM HEPES, and 5.5 mM glucose, pH 7.4) and 140 mM KCl were prepared before experimentation and stored at 4°C.

**Myographic studies.** Vascular responses to OT were studied using an isometric myograph system. Small mesenteric arteries, uterine arcuate arteries (averaging 250 μm in diameter), and thoracic aorta (for comparative purposes) from pregnant and nonpregnant Sprague-Dawley rats were placed in a vessel bath containing 5.0 ml of HEPES buffer maintained at 37°C and mounted on the wire myograph system. Vessels were then given a 30-min recovery period and replenished with fresh HEPES buffer every 10 min. Four baths in the myograph system allowed for parallel experiments to be conducted on four arteries from the same animal. Cumulative doses of phenylephrine (1 to 50 × 10⁻⁶ M) were administered to all arteries to determine individual EC₅₀ concentrations for each preparation. The vessels were then given another 30-min recovery period.

A crossover experimental design was used to study the response of the vessels to cumulative doses of OT (10⁻¹⁴ to 10⁻⁶ M). Two vessel types were studied each day (2 small mesenteric arteries and 2 uterine arcuate arteries), allowing for a time control and an experimental vessel for each vessel type. Experimental vessels and time control vessels were preconstricted with their EC₅₀ concentration of phenylephrine to establish a baseline from which subsequent relaxation or constriction responses could be measured. The amount of force produced from the EC₅₀ dose of phenylephrine was set as 100%, and subsequent responses were normalized to this. After completion of the OT concentration-response curve and a 30-min recovery period, the time control vessel from the previous experiment became the experimental vessel, and vice versa.

The studies involving the vasopressin V₁a receptor antagonist were performed using small mesenteric vessels and a crossover experimental design. One vessel of the pair was preincubated with the specific AVP V₁a receptor antagonist (10⁻⁶ M) for 20 min while the control received only HEPES buffer. Both vessels were then preconstricted to their EC₅₀ dose of phenylephrine, and cumulative doses of OT (10⁻¹⁴ to 10⁻⁶ M) were administered to produce a concentration-response curve. After completion of the OT concentration-response curve and a 30-min recovery period, the OT control vessel from the previous experiment became the experimental vessel, and vice versa.

To ensure all vessels had intact endothelial layers, a bolus dose of methacholine (10⁻⁶ M) was administered to each vessel, and relaxation responses were documented. At the end of each experiment, 5.0 ml of 140 mM KCl were administered to the vessels to ensure their ability to constrict.

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**RESULTS**

There was no vasorelaxation observed in response to OT in the rat small mesenteric artery (Fig. 1). A significant vasoconstrictor response was observed in mesenteric arteries from both nonpregnant and pregnant rats at OT concentrations >10⁻⁸ M. The vasoconstrictor response in the vessels from pregnant animals was significantly greater than in vessels from the nonpregnant group. In the uterine arcuate artery (Fig. 2) there was no vasorelaxation response but a vasoconstrictor response at OT concentrations >10⁻⁹ M. There was no significant difference between vessels from the nonpregnant and pregnant animals. There was no response to OT in the aorta from the nonpregnant animals (Fig. 3). There was a statistically significant vasoconstrictor response in the aorta from the pregnant animals at the highest concentration of OT.

![Fig. 1. Responses to oxytocin (OT) of preconstricted small mesenteric arteries from both nonpregnant and pregnant Sprague-Dawley rats. The vasoconstrictor response in the vessels from pregnant animals was significantly greater than in vessels from the nonpregnant group.](http://ajpheart.physiology.org/)
two-way ANOVA, there was no statistically significant difference in the responses between the aortas from the nonpregnant and pregnant animals.

In the experiments using coincubations of OT with a specific vasopressin V1a receptor antagonist with mesenteric vessels from nonpregnant rats, the significant vasoconstrictor response at the highest concentration of OT was completely blocked by the vasopressin V1a antagonist (Fig. 4).

Arteries from three to five animals were pooled to isolate RNA from the vascular beds. mRNA for OTR was detected in nonpregnant small mesenteric artery, uterine arcuate artery, and thoracic aorta (see insets in Figs. 1–3). Because of the small amounts of RNA isolated from these tissues, the yield of RNA could not be quantified, and no effort has been made to make quantitative comparisons after RT-PCR.

**DISCUSSION**

This is the first systematic study of the effects of OT in concentrations that encompass the physiological range (at least in plasma) and compares relevant vascular beds in pregnant and nonpregnant females. Although we have demonstrated the presence of mRNA for OT in resistance-sized arteries from both pregnant and nonpregnant animals, OT failed to elicit a vasorelaxation response in any of the vessels studied. This is in agreement with earlier observations using a pressurized myograph system with mesenteric arteries from male rats (38) or small uterine resistance arteries from nonpregnant rats (8). Similar observations were made using a wire myograph system with guinea pig uterine vessels (26). However, in these previous studies, only high concentrations of OT (≥10⁻⁶ M) were used. Circulating concentrations of OT in either the pregnant or nonpregnant state are less than 10⁻¹⁰ M in both rat (21) and human (16, 44). In our studies, there was no vasorelaxant response at any concentration as low as 10⁻¹⁴ M. In other studies (data not shown), we measured no effect of OT with concentrations as low as 10⁻²⁵ M.

Several in vitro studies have provided support for a possible direct vasorelaxation response to OT. Thiessoni et al. (42) demonstrated binding of radiolabeled OT to cultured human umbilical vein endothelial cells and confirmed, using pharmacological and molecular biological approaches, that this binding was mediated by specific OT receptors on these cells. Furthermore, they showed that OT stimulated phosphatidylinositol turnover, mobilization of intracellular Ca²⁺, activation of the cGMP pathway, and increased production of nitric oxide. Using an isometric muscle bath preparation with strips of vessel wall from term human umbilical artery and vein, Altura et al. (2) found only a vasoconstrictor effect. In isolated canine cerebral vessels, OT causes a vasorelaxation response that appears to be mediated through vasopressin V1a receptors and is endothelium dependent (27). Our inability to demonstrate a vasodilatory response to OT in
the small mesenteric and uterine arcuate arteries may reflect the marked variation of responses to vasoactive substances in different vascular beds (1, 5, 12).

Subcutaneous or intravenous administration of OT to rats causes a brief increase in mean arterial pressure accompanied by bradycardia and decreased cardiac output leading to a prolonged decrease in blood pressure (32–34). With intracerebroventricular injection of OT, the initial increase in blood pressure was not observed (32). This could be explained by an initial peripheral effect of OT on the vasculature causing hypertension followed by a slower central nervous system effect causing a decreased blood pressure. Oxytocinergic neurons project from the paraventricular nucleus into areas known to be important for cardiovascular control, and electrical stimulation of these nuclei decreased blood pressure through the inhibition of sympathetic preganglionic neurons (46). Although only a small fraction of systemically administered OT crosses the blood-brain barrier, this could be sufficient to stimulate a central response causing reduced blood pressure (24).

Jankowski and colleagues (22, 23) have suggested another potential mechanism through which OT may act. They have identified OT synthesis and OT binding sites in the rat aorta and vena cava and demonstrated the presence of mRNA for OT and OTR in the rat aorta, vena cava, and the pulmonary vasculature (22, 23). They also demonstrated that OT can be produced and secreted by cardiac myocytes and that treatment of these cells with OT stimulates release of atrial natriuretic peptide that could decrease the force and rate of cardiac contraction and decrease mean arterial pressure (19). Additionally, physiological doses of OT that enhance glomerular filtration rate induce natriuresis (11), and this could contribute to lowering of blood pressure.

Our finding of a vasoconstrictor response in the small mesenteric and uterine arcuate vessels with high concentrations of OT is similar to previous reports (8, 26, 38). In our studies, the ability of the vasopressin V1a antagonist to completely block this effect strongly suggests the vasoconstrictive actions were mediated through the V1a receptor rather than OTR. This is in keeping with previous findings using rat aorta (38) and uterine artery from rat (8) or guinea pig (26). The sensitivity of these vessels to vasopressin was two to three orders of magnitude greater than to OT. Additionally, a specific OT receptor antagonist failed to counteract the vasoconstrictor effect of OT (8, 38). In our experiments using the V1a antagonist (Fig. 4), the constriction response to OT appeared to be a log dose weaker than in the other experimental protocols. However, this may have resulted from the crossover design wherein the OT-treated artery in the second arm had been exposed to the V1a antagonist in the first arm of the protocol. It is possible that the antagonist had not been completely removed in the intervening 30-min washout period. Alternatively, the apparent shift in sensitivity to OT could be a result of V1a receptor internalization (3) during the first “control” period.

A significant decrease in vascular response to pressor agents during pregnancy is well documented (17, 29, 30, 39, 40, 45). Our finding of an increased vasoconstrictor responsiveness to OT in the small mesenteric arteries from pregnant compared with nonpregnant animals is interesting. This would be in agreement with the studies of Jovanovic et al. (25) who noted an increased endothelium-dependent vasoconstrictor response to vasopressin in uterine arteries from pregnant compared with nonpregnant guinea pigs. However, they also found a decreased responsiveness to OT in similar preparations even though they concluded that the OT effect was mediated through vasopressin V1a receptors. Using a pressurized myograph system, St-Louis et al. (39) demonstrated a decreased responsiveness of rat mesenteric vessels to vasopressin during pregnancy. The reason for these discrepancies is not clear but may be related to differences in species, in vascular beds (1, 5, 12), or in methodologies. In any case, it is likely that this response is pharmacological and of little physiological relevance.

Our RT-PCR results confirm the findings of Jankowski et al. (23) in the aorta and also demonstrate the presence of mRNA for OTR in the mesenteric and uterine vessels of the rat. Because of the small amount of tissue in these latter vessels, we were unable to measure OTR protein levels. However, the findings of others suggest that functional OTR are present in vascular tissues (4, 19, 22, 41, 42). Our results suggest they are not responsible for a direct physiological effect on vascular tone in the mesenteric or uterine arterial systems.

In conclusion, our results indicate that OT does not have a direct physiologically significant effect on vascular tone in the resistance-sized vessels from nonpregnant or pregnant rats. If OT is an important mediator of vascular control, these data suggest that its actions are indirect. We have confirmed the presence of mRNA for OTR in the rat aorta and have demonstrated its presence in resistance-sized vessels including the small mesenteric and uterine arteries. Its physiological function, if any, remains unknown. Future studies are required to determine a role of OT or its receptor in maternal adaptation to pregnancy.

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