

# Negative Inotropic and Chronotropic Effects of Oxytocin

Suhayla Mukaddam-Daher, Ya-Lin Yin, Josée Roy, Jolanta Gutkowska, René Cardinal

**Abstract**—We have previously shown that oxytocin receptors are present in the heart and that perfusion of isolated rat hearts with oxytocin results in decreased cardiac flow rate and bradycardia. The mechanisms involved in the negative inotropic and chronotropic effects of oxytocin were investigated in isolated dog right atria in the absence of central mechanisms. Perfusion of atria through the sinus node artery with  $10^{-6}$  mol/L oxytocin over 5 minutes (8 mL/min) significantly decreased both beating rate ( $-14.7 \pm 4.9\%$  of basal levels,  $n=5$ ,  $P<0.004$ ) and force of contraction ( $-52.4 \pm 9.1\%$  of basal levels,  $n=5$ ,  $P<0.001$ ). Co-perfusion with  $10^{-6}$  mol/L oxytocin receptor antagonist ( $n=3$ ) completely inhibited the effects of oxytocin on frequency ( $P<0.04$ ) and force of contraction ( $P<0.004$ ), indicating receptor specificity. The effects of oxytocin were also totally inhibited by co-perfusion with  $5 \times 10^{-8}$  mol/L tetrodotoxin ( $P<0.02$ ) or  $10^{-6}$  mol/L atropine ( $P<0.03$ ) but not by  $10^{-6}$  mol/L hexamethonium, which implies that these effects are neurally mediated, primarily by intrinsic parasympathetic postganglionic neurons. Co-perfusion with  $10^{-6}$  mol/L NO synthase inhibitor (L-NAME) significantly inhibited oxytocin effects on both beating rate ( $-1.85 \pm 1.27\%$  versus  $-14.7 \pm 4.9\%$  in oxytocin alone,  $P<0.05$ ) and force of contraction ( $-24.9 \pm 4.4\%$  versus  $-52.4 \pm 9.1\%$  in oxytocin alone,  $n=4$ ,  $P<0.04$ ). The effect of oxytocin on contractility was further inhibited by L-NAME at  $10^{-4}$  mol/L ( $-8.1 \pm 1.8\%$ ,  $P<0.01$ ). These studies imply that the negative inotropic and chronotropic effects of oxytocin are mediated by cardiac oxytocin receptors and that intrinsic cardiac cholinergic neurons and NO are involved in these actions. (*Hypertension*. 2001;38:292-296.)

**Key Words:** receptors ■ neurons ■ heart rate ■ contractility ■ nervous system, parasympathetic ■ nitric oxide

Oxytocin, 1 of 2 major posterior pituitary hormones (oxytocin and vasopressin), is a neuropeptide synthesized primarily in magnocellular neurons in the paraventricular and supraoptic nuclei of the hypothalamus, which project to posterior pituitary, median eminence, and several brain regions. Oxytocin is also produced in peripheral tissues,<sup>1</sup> including the heart. We have recently shown that the heart is an important source of oxytocin production and synthesis.<sup>2</sup>

In addition to its well-known effects on reproductive functions, such as uterine contraction, milk-ejection reflex, and induction of maternal behavior,<sup>3,4</sup> oxytocin was recently shown to be involved in endocrine and neuroendocrine regulation through receptor-mediated actions exerted on the heart, vasculature, and kidneys.<sup>5-9</sup> In humans, intravenous oxytocin injections cause biphasic dose-dependent changes in mean arterial pressure that consist of an initial pressor response accompanied by bradycardia and a decrease in cardiac output, followed by a prolonged fall in mean arterial pressure accompanied by increased cardiac output.<sup>10</sup> Oxytocin administration at the rat upper spinal cord<sup>11</sup> or into acutely decentralized canine stellate or middle cervical ganglia<sup>12</sup> induces significant increases in cardiac rate and force. Oxytocin perfusion of isolated rat hearts results in dose-dependent negative chronotropic effect while exerting positive inotropic

effect.<sup>13</sup> Rosaeg et al,<sup>14</sup> however, did not demonstrate any effects on contractile force in human atrial trabeculae. We have shown that isolated rat heart perfusion with oxytocin induces significant bradycardia.<sup>5</sup>

The hypotensive and bradycardic effects of oxytocin in whole animals could be centrally or peripherally mediated. In our studies of perfused isolated rat heart,<sup>5</sup> oxytocin resulted in bradycardia in the absence of central mechanisms. However, although decentralized, isolated cardiac preparations are by no means denervated but have an elaborate intrinsic nervous system<sup>15</sup> that is capable of participating in cardiac regulation.<sup>16</sup> Parasympathetic efferent postganglionic neurons, when activated either chemically or electrically, suppress atrial rate and force, atrioventricular nodal conduction, and ventricular contractile force. Parasympathetic postganglionic neurons in each intrinsic cardiac ganglionated plexus innervate tissues throughout the heart.<sup>17</sup> The cell bodies of intracardiac neurons exist as clusters located in several intrapericardial and epicardial ganglionated plexuses. In particular, the right atrial ganglionated plexus, which is contained within a triangular fat pad located on the ventral aspect of the right atrial free wall, has been postulated to be the main relay for selective parasympathetic innervation of the sinus node,<sup>18-20</sup> although efferent projections to other atrial areas also course

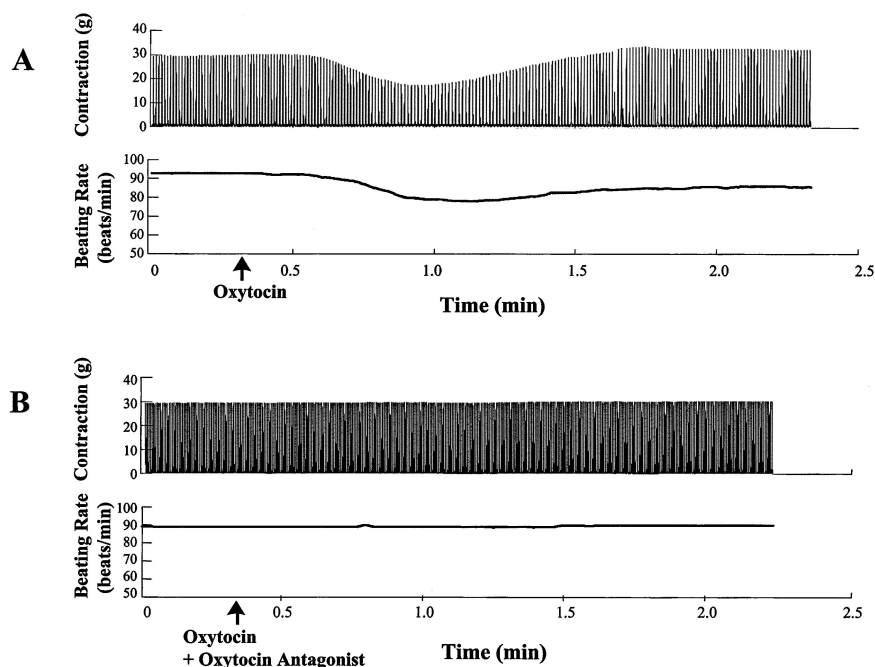
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**Figure 1.** Representative tracing of effect of oxytocin perfusion ( $10^{-6}$  mol/L) through the sinus node artery on right atrial contractility and beating rate (A) and inhibition by oxytocin receptor antagonist (B).

through this structure.<sup>21</sup> Therefore, oxytocin may reduce the force and frequency of contraction by directly acting on intrinsic cardiac neurons.

Accordingly, the following studies were performed to investigate the mechanisms involved in the negative chronotropic and inotropic effects of oxytocin. Beating rate and force of contraction were measured on isolated dog right atrial tissue superfused in vitro with oxytocin and various inhibitors.

## Methods

### Tissue Preparation

Animal and tissue preparation was performed as previously detailed,<sup>20</sup> following the Guidelines of the Canadian Council on Animal Care, and was approved by our institution. In brief, healthy adult mongrel dogs of either gender (weight, 25 to 30 kg) were anesthetized with thiopental sodium (25 mg/kg IV) and artificially ventilated with room air. The heart was exposed through a left thoracotomy, and the pericardium was opened. One minute after heparin (1000 IU) injection into the left ventricle, the heart was excised and quickly immersed in cold Tyrode solution. Right atrial preparations consisting of the anterior right atrial free wall and adjacent sinus node region, the atrial appendage, and the posterior right atrial wall, including right pulmonary vein tissue and adjacent left atrial tissue, were isolated. The sinus node artery was cannulated with polyethylene tubing (PE50). The tissue was pinned to the Silastic floor of a tissue bath with the endocardium facing up. The preparations were superfused with oxygenated Tyrode's solution by gravity (20 mL/min) and perfused via the cannula by a peristaltic pump at a constant flow of 8 mL/min. The Tyrode's solution ([in mmol/L] NaCl 128, KCl 4.7, MgSO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 0.47, NaH<sub>2</sub>PO<sub>4</sub> 0.5, CaCl<sub>2</sub> 2.3, and dextrose 11.1; pH 7.4) was gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub> and kept at 37°C. The superior part of the atrial preparation was connected to an isometric force transducer by a silk thread. A baseline tension of  $7.6 \pm 1.2$ g was applied and maintained throughout the experiment. One pair of bipolar silver electrodes was brought to contact with the surface of the preparation. The other pair was connected to a Nihon Kohden amplifier to record the sinus rate.

### Electrophysiological Studies

The inotropic and chronotropic effects of oxytocin were investigated as follows. After a 40-minute stabilization period, oxytocin was administered at increasing concentrations ( $10^{-11}$  to  $10^{-6}$  mol/L). The dose of  $10^{-6}$  mol/L was shown to be most effective, and therefore, oxytocin at  $10^{-6}$  mol/L was used in the subsequent studies.

Oxytocin ( $10^{-6}$  mol/L) was administered alone or with inhibiting drugs. Specificity of the response was determined by use of a selective oxytocin receptor antagonist. The mechanisms involved in the effects were evaluated by inhibiting nerve-related activity with tetrodotoxin ( $5 \times 10^{-8}$  mol/L), the nicotinic cholinergic blocking agent hexamethonium ( $10^{-6}$  mol/L), the muscarinic cholinergic receptor blocker atropine ( $10^{-6}$  mol/L), and the NO synthase inhibitor *N*<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME;  $10^{-6}$  and  $10^{-4}$  mol/L). All inhibitors were given before oxytocin via the perfusion medium over a period of 5 to 10 minutes (time at which steady-state response was established).

### Drugs

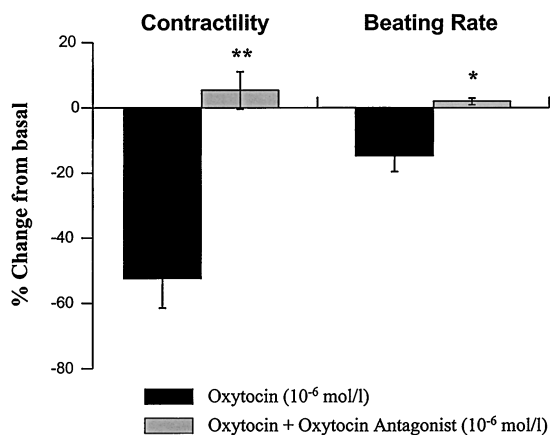
Hexamethonium and tetrodotoxin were purchased from Sigma, atropine sulfate from BDH Chemicals Ltd, L-NAME from RBI Sigma-Aldrich Canada, and oxytocin receptor antagonist [d(CH<sub>2</sub>)<sup>5</sup>Tyr(OMe)<sup>2</sup>,Thr<sup>3</sup>,Tyr<sup>9</sup>-NH<sub>2</sub>] OVT (compound VI) from Peninsula Laboratories. All compounds are water soluble.

### Statistical Analysis

The cardiac responses elicited in each atrium were evaluated by comparison of the parameters measured immediately before each intervention and maximal changes elicited after each intervention. These changes are reported as percent difference from control basal values (100%). Data obtained with oxytocin plus inhibitors were expressed as percent of those obtained with oxytocin alone. Significant differences between baseline and effects of oxytocin were tested by paired Student's *t* test, whereas differences between the effects of oxytocin with and without inhibitors were tested by nonpaired Student's *t* test with the RS/1 computer program.  $P < 0.05$  was considered significant. All data are reported as mean  $\pm$  SEM.

## Results

Perfusion of oxytocin directly into the sinus node artery of 5 isolated right atrial preparations resulted in a transient but

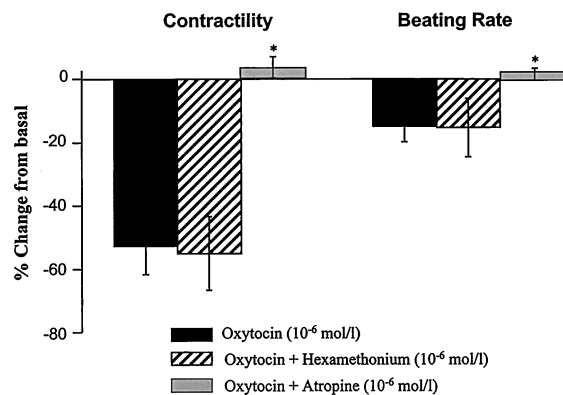


**Figure 2.** Reduction of beating rate and force of contraction by oxytocin ( $10^{-6}$  mol/L) perfusion through the sinus node artery of isolated right atria ( $n=5$ ), presented as percent decrease from baseline, and total inhibition of effects by co-perfusion with  $10^{-6}$  mol/L oxytocin receptor antagonist ( $n=3$ ). Values are expressed as mean  $\pm$  SEM. \* $P<0.04$ ; \*\* $P<0.004$  vs oxytocin alone.

significant decrease in both beating rate and force of contraction. The effects developed rapidly, beginning within 40 seconds, which corresponded to transit time through the dead space of the tubing. The effects were transient, reaching a minimum then returning to the basal value within 80 seconds despite continuation of perfusion (Figure 1). Figures 1A and 2 show that beating rate decreased from a baseline value of  $98.2 \pm 7.1$  to  $85.0 \pm 10.7$  bpm or  $-14.7 \pm 4.9\%$  below baseline ( $P<0.004$ ), whereas the amplitude of contractility decreased from a baseline value of  $27.0 \pm 3.7g$  to  $13.8 \pm 3.7g$ , representing a decrease of  $52.3 \pm 9.1\%$  below baseline ( $P<0.001$ ). When infusion was repeated after a 30-minute washout period, the rate and contractile responses were of a lower magnitude. Therefore, only results obtained from first treatment per atrium are reported.

Perfusion with oxytocin antagonist alone did not have any effect on heart rate and force of contraction. However, coadministration with oxytocin antagonist ( $n=3$ ) completely inhibited the effects of oxytocin on beating rate ( $2 \pm 1\%$ ,  $P<0.04$  versus oxytocin alone) and force ( $P<0.004$  versus oxytocin alone), which implies that these effects are mediated by oxytocin receptors (Figures 1B and 2). Co-perfusion with tetrodotoxin ( $n=3$ ) also inhibited the negative effects of oxytocin on spontaneous beating rate ( $-0.85 \pm 0.61\%$ , versus  $-14.70 \pm 4.90\%$ ,  $P<0.02$ ) and force of contraction ( $-4.45 \pm 1.25$  versus  $-52.36 \pm 9.08$ ,  $P<0.02$ ), which suggests that these effects are neurally mediated.

Figure 3 shows that the reduction in beating frequency and force by oxytocin persisted after co-perfusion with hexamethonium ( $-15.2 \pm 9.2\%$  and  $-54.8 \pm 11.6\%$ , respectively;  $n=5$ ), which implies that the effects were not dependent on nicotinic cholinergic transmission. However, coadministration with atropine ( $n=6$ ) totally eliminated the effects of oxytocin on beating frequency ( $P<0.001$  versus oxytocin alone) and force ( $P<0.001$  versus oxytocin alone). These results indicate that these effects are mediated by acetylcholine (ACh) release at cardiac parasympathetic postganglionic neurons.

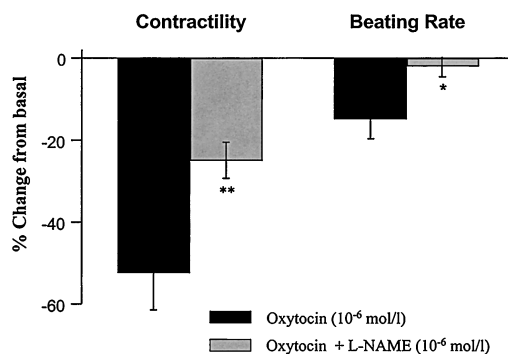


**Figure 3.** Inhibition of oxytocin effects on beating rate and force of contraction by  $10^{-6}$  mol/L atropine ( $n=6$ ) but not  $10^{-6}$  mol/L hexamethonium ( $n=5$ ). \* $P<0.001$  vs oxytocin alone.

The addition of L-NAME, an NO synthase inhibitor, significantly inhibited the negative inotropic and chronotropic effects of oxytocin in 4 of 5 preparations. In these 4 preparations, co-perfusion with L-NAME (Figure 4) resulted in a  $1.85 \pm 1.27\%$  decrease in beating rate versus a  $14.7 \pm 4.9\%$  decrease by oxytocin alone ( $P<0.05$ ) and a decrease in contractility by  $24.9 \pm 4.4\%$  versus  $52.3 \pm 9.1\%$  with oxytocin alone ( $P<0.04$ ). However,  $10^{-4}$  mol/L L-NAME did not have a further effect on beating rate but further inhibited the oxytocin effect on contractility to  $-8.1 \pm 1.8\%$  ( $P<0.01$ ,  $n=3$ ). Inhibition of the negative inotropic and chronotropic effects of oxytocin by L-NAME indicates that NO is involved in these actions.

## Discussion

The present study shows that in vitro superfusion and perfusion of isolated dog right atria with oxytocin lowers both the force and frequency of contraction. The effects are mediated by specific oxytocin receptors in the right atrium, completely blocked by tetrodotoxin and atropine but not hexamethonium, and inhibited by NO synthase inhibitor. These studies imply that oxytocin receptors in the heart may be localized on intrinsic cholinergic neurons, and that on activation, they release ACh to decrease heart rate and force of contraction. NO is involved in these actions.



**Figure 4.** Inhibition of oxytocin effects on beating rate and force of contraction by  $10^{-6}$  mol/L L-NAME ( $n=4$ ). \* $P<0.05$ ; \*\* $P<0.04$  vs oxytocin alone.

Several mechanisms may be involved in oxytocin actions on the heart. We have previously localized oxytocin and oxytocin receptors to atrial cardiomyocytes and shown that oxytocin perfusion stimulates the release of atrial natriuretic peptide (ANP).<sup>5</sup> ANP may act on its receptors in the heart to increase cGMP production, resulting in reduced force of contraction and bradycardia. Under the conditions of the present experiments, however, the addition of ANP to the perfusion buffer increased the force of contraction and had no effect on beating rate<sup>22</sup>; thus, ANP may not be involved.

On the other hand, the present results in isolated right atria clearly show that oxytocin effects on beating rate and force of contraction are totally blocked by the muscarinic blocking drug atropine. Because atropine suppresses transmission from the postganglionic fiber to the cardiac effector cells, these results imply that oxytocin may primarily act on oxytocin receptors present on parasympathetic postganglionic fibers to stimulate the release of ACh, which acts on muscarinic receptors and results in lower rate and force of contraction. This study does not rule out the presence of oxytocin receptors on sympathetic fibers; in particular, blockade with atropine unmasked a possible slight positive effect that may be elicited by sympathetic activation.

Activation of cardiac muscarinic receptors may influence cardiac functions through regulation of the activity of several ion channels. Muscarinic receptor activation inhibits cardiac L-type calcium channel ( $I_{CaL}$ ) through interaction with G proteins.<sup>23</sup> This inhibition is mediated in part via activation of NO synthase and generation of NO, which stimulates soluble guanylyl cyclase to produce cGMP<sup>24</sup> and subsequent activation of cGMP-dependent protein kinase G and dephosphorylation of potassium and calcium channel proteins. Also, cGMP activates a phosphodiesterase, which decreases cAMP concentration.<sup>25</sup> In addition, direct inhibition of adenylyl cyclase and cAMP production via  $G_i/G_o$  subunits or activation of phosphatases by muscarinic receptors may contribute substantially to the antiadrenergic effect on  $I_{CaL}$ .<sup>24,25</sup>

Most important, however, is the ACh activation of muscarinic receptors in the sinoatrial nodal tissue that may result in opening of potassium channels and membrane hyperpolarization, thus very quickly (relative to the slow effect expected by second-messenger pathways) decreasing beating rate and force of contraction. ACh may interact with ACh-regulated  $K^+$  channels,  $I_{K(ACh)}$ , and the  $K^+$  channels that conduct the transient outward current,  $I_{to}$ , both of which are abundant in atrial cell membranes.<sup>26,27</sup> Activation of these 2 channels will act to diminish the duration of the atrial action potential. This in turn will diminish  $Ca^{2+}$  influx into atrial myocytes and thereby diminish atrial contractility.<sup>27,28</sup>

The quick and transient oxytocin effect to reduce atrial rate and contractility is similar to that observed with ACh. ACh is synthesized mainly in the nerve endings of postganglionic neurons and stored in vesicles near the site of subsequent release.<sup>29</sup> On nervous stimulation, the stored ACh is released, and then it diffuses across the neuroeffector gap and interacts with muscarinic receptors on the membrane of the effector cell. Therefore, rapid hydrolysis of ACh, depletion of ACh stores, or desensitization of receptors may influence the rapidity of the loss of effects during perfusion and after

repeated perfusion with oxytocin. It is possible that in excised superfused preparations, ACh stores are more vulnerable than in situ and that ACh released from the nerve ending is rapidly hydrolyzed to acetate and choline by acetylcholinesterase, which is abundant in atrial and nodal tissues.<sup>29</sup> On the other hand, it appears less likely that desensitization of either muscarinic or oxytocin receptors is involved, because muscarinic-mediated bradycardia could be maintained for a prolonged time and because in our previous studies in isolated rat hearts,<sup>5</sup> the oxytocin effect on ANP release persisted throughout a continuous perfusion over 25 minutes.

Because these experiments were performed in isolated right atrial preparations, we postulate that in addition to cardiomyocytes,<sup>2</sup> oxytocin receptors may be primarily localized on intrinsic cholinergic neurons. These cardiac neurons are capable of generating spontaneous activity independently of inputs from central neurons and other intrathoracic neurons, and their activity can be modified by both intracardiac and extracardiac afferent inputs.<sup>30,31</sup> In support of this, Armour and coworkers<sup>32</sup> have shown that direct microinjection of oxytocin into active canine intrinsic cardiac neurons induced increases and decreases in neural activity depending on the population of neurons studied.

In summary, we have previously shown that oxytocin and oxytocin receptors are present and synthesized in the rat heart. We now provide evidence that oxytocin is a neuro-modulator involved in the nervous control of the heart. Oxytocin may act in an autocrine/paracrine manner to modulate the release of ACh from intrinsic cardiac cholinergic neurons.

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