

Full Length Research Paper

Oxytocin ameliorates the deleterious effect of pain in adult male rats

Nessren M. Abd el-Rady^{1*}, Omyma G. Ahmed¹, Ebtihal A. Abd El-Aziz¹, Ayman M. Osman² and Amel Taha³

¹Department of Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt.

²Department of Anesthesia and Intensive Care, Faculty of Medicine, Assiut University, Assiut, Egypt.

³Department of Histology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Received 18 August, 2016; Accepted 14 September, 2016

Oxytocin (OXT) is a neuropeptide with a peripheral role in reproduction, though it may have a central effect. The aim of the study is to verify its central pathophysiological mechanisms on experimental rats. Rats were divided into control, OXT (which divided into intrathecal OXT and IV OXT subgroups), and antagonist group: OXT and l-glutamate Na⁺ subgroup with measuring of pain threshold, cardiovascular changes, and pain related mediators (OXT, serotonin, and dopamine) and histopathological examination. A significant increase of the pain threshold in rats was observed in intrathecal oxytocin subgroup. The response was absent in IV oxytocin subgroup. The analgesic effect of OXT contributes to direct increase of OXT receptors maker in different brain areas and spinal cord and through glutamergic pathway. Besides, OXT induces significant increase in the levels of neurotransmitters related to the pain in serum and brain homogenate of this experimental study. Administration of OXT can counterbalance the pain troubles without major detectable detrimental effects on cardiovascular system.

Key words: Oxytocin (OXT), neuropeptide, cardiovascular system, glutamergic pathway, serotonin and dopamine.

INTRODUCTION

Oxytocin (OXT) is a hormone synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) to travel into two pathways (Balki et al, 2014). Either, from the posterior pituitary to the hypophyseal blood stream to enter the peripheral blood for its hormonal action, or OXT transported in axons and released in target brain areas (e.g., hippocampus, amygdala, striatum, hypothalamus, nucleus accumbens, and midbrain) (Moreno et al., 2013).

For many years, OXT was widely known for its peripheral role in milk letdown and uterine contractions (Baskerville and Douglas, 2010). However, great interest is taken nowadays concerned about the role of OXT in pain modulation, especially because it is a natural hormone and released normally under various conditions (Yang et al, 2011b) however, its analgesic action is debated. In this study, we clarified the analgesic role of oxytocin; hope to understand the mechanism of OXT in

*Corresponding author. Email. drnessren82@yahoo.com

modulating pain process in experimental rats.

MATERIALS AND METHODS

The experimental study

During all the experiments, the animals were treated in accordance with the published rules for animal laboratory care, and the protocol has been approved by local Ethical Committee for Scientific Research at Faculty of Medicine, Assiut University, Egypt.

Rats and maintenance

Twenty-four adult male Wistar Albino rats, weighing (180 to 220 g), were housed in clean spacious cages (up to 4 per cage) in animal house of Faculty of Medicine, Assiut University. They were maintained on a natural 12:12-h light-dark cycle in an aerated room, temperature ($25 \pm 5^\circ\text{C}$), food (standard rat pellets) and water available *ad libitum*. The animals were habituated for handling and the testing environments for four days.

Experimental design

The experimental animals were divided into three main groups. (1) Control group: Rats received intrathecal saline; (2) The OXT group (A: Rats injected with single 3.12 ng OXT/10 μl saline intrathecally (Yang et al, 2011b); B: Rats received the same dose of OXT once intravenously), and (3) The antagonist group (C: rats injected by single dose of 0.32 μM l-glutamate sodium /1 μl saline intrathecally (Yang et al, 2006) followed by intrathecal OXT).

Laboratory methods

Measurement of pain threshold

It was done individually in animals of all groups before and after administration of the analgesic measure. These tests include the following:

Tail-flick test: Each rat was placed in a restrainer and the distal third of its tail positioned unrestricted at the point of focus of a noxious radiant heat source (a focused projector bulb). The period from heat application to tail-flick until the animal withdraws the tail was recorded (Silva et al, 2013).

Hot plate test to noxious heat: Pain reflexes in response to a thermal stimulus were measured using Hot Plate Analgesia Meter (Model 30 socrel, Ugo Basile biological research apparatus, Comerio-Va-Italy). The hot plate surface was heated to a constant temperature of $55 \pm 0.5^\circ\text{C}$ (A temperature at which both A- and C-fibers are activated (Guyton, 2010), as measured by a built-in digital thermometer.

Animals were placed on the hot plate (30 cm in diameter), which was surrounded by a clear acrylic cage (25 cm tall, open top). The time until animal pain reflex appear (hindpaw lick) was measured (Adrienne et al, 2013).

Measurement of blood pressure

It was measured over rat-tail by LE 5001 Pressure Meter (Panlab HARVARD apparatus, LE5001 AC-110/220V 18W, 50/60 Hz F0, 2A L250V) made in Spain.

Measurement of heart rate

It was measured by ECG needle electrodes, which were inserted under skin of gently extended limbs of the supine animals. ECG was recorded at paper speed of 50 mm/s. (AD Instruments Pty Ltd, Castle Hill, Australia). Sensitivity was adjusted so as 1 mV causes 1 cm displacement of the baseline.

Biochemical assays

2 mL blood was collected from the retro-orbital venous plexus immediately before scarifying the animals. The whole blood incubated to clot for 30 min in clean screw-capped polypropylene tubes at room temperature and the serum separated by centrifugation at 3000 rpm for 10 min. The separated serum was aliquotted and stored frozen in Eppendorf's tubes at -20°C for assessment of the serum level of OXT, serotonin and dopamine by ELISA kits.

B-Brain homogenate preparation

At the end of the experiment, animals were sacrificed by ether and perfused intracardially with saline and 10% neutral buffered formalin. The brain and spinal cord were extracted from the skulls and vertebral column. They were mid-sagittally divided into two portions. The first portion was kept frozen at -20°C until use. Then this portion was weighed and placed in empty glass tubes per 1 g of tissue then homogenized with phosphate buffer in a motor-driven homogenizer. The homogenate was centrifuged at 2,800 g for 10 min at 4°C then the supernatant was placed in labeled vials and stored at -80°C for measurement of OXT, serotonin and dopamine by enzyme-linked immunosorbent assays (ELISA) with monoclonal antibodies against each substance according to manufacturer's protocol.

Histopathological examination

The frontal cortex was dissected from the remaining portion of the brain (by coronal cut rostral to the corpus callosum) and the spinal cord, then the sections was used for immunohistochemistry to illustrates the presence of Chromogranin positive cells that indicates the presence of OXT receptors .

Data analysis

Statistical analysis was carried out using SPSS (version 20). Data were analyzed non-parametrically (due to the small number of animals in the groups and the cut-off time of latency performance). Data were expressed in mean \pm SD. First, to compare the difference between pre-and post-values using the Wilcoxon Signed Ranks Test. Second, to compare the differences between the control and other groups, the Mann/Whitney U-test was used. The level of statistical significance was $P < 0.05$ for all statistical evaluations.

RESULTS

Effect of administration of the analgesic measure on pain threshold in different studied group

Statistical analysis showed a significant increase in the pain threshold in the rats after administration

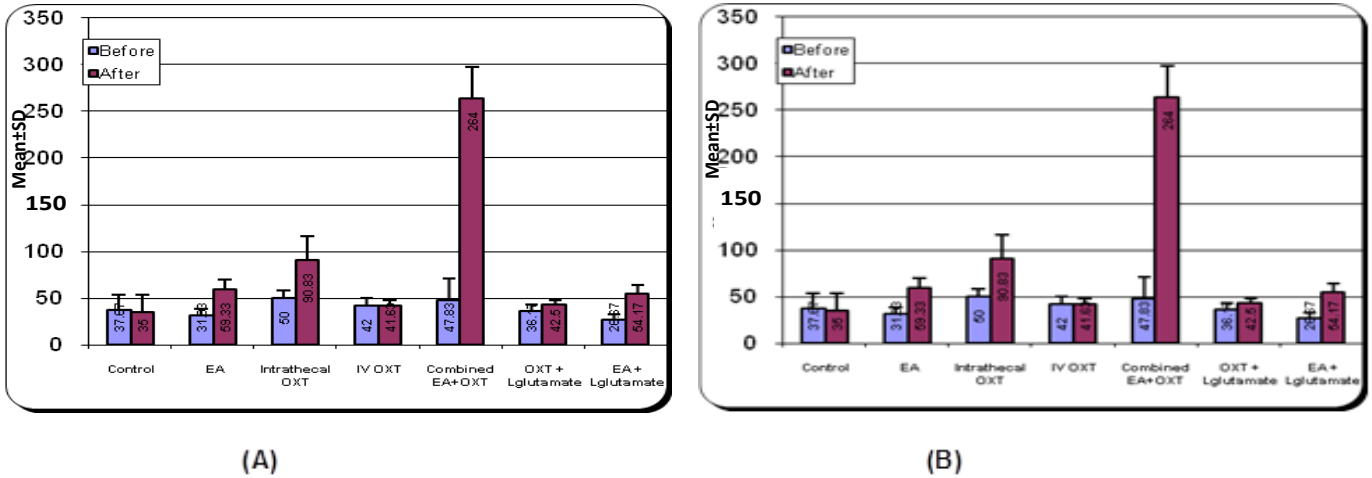


Figure 1. Comparison pre- and post-values of tail flick test (A) and hot plate test (B) in different studied groups of rats. Values represent mean ±SD. OXT = oxytocin.

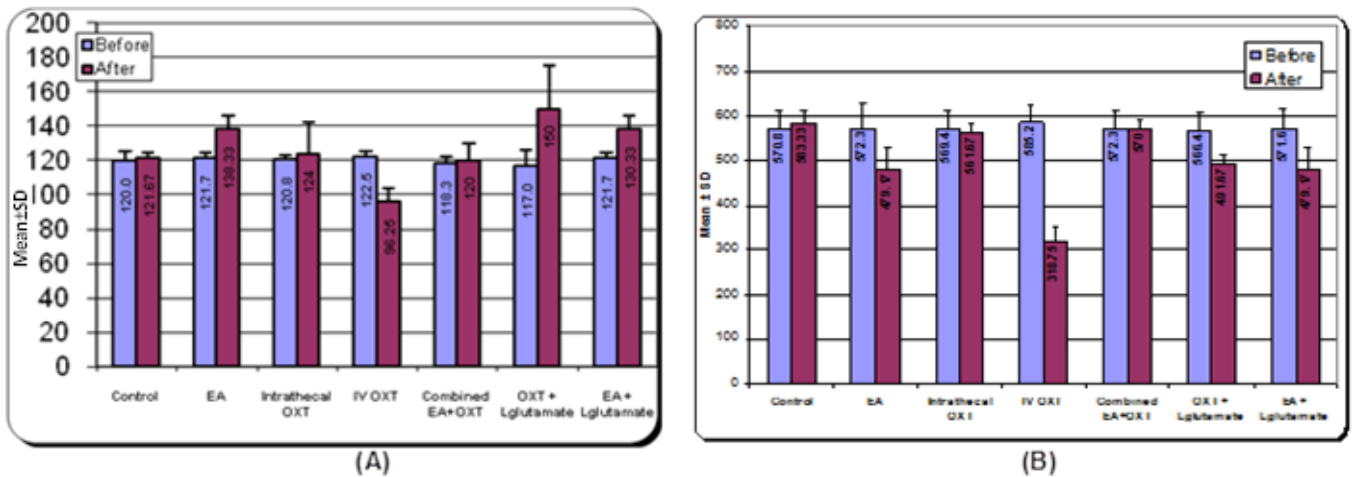


Figure 2. Comparing pre- and post-values of ABP (A) and HR (B) in different studied groups of rats. Values represent mean ±SD. OXT = oxytocin.

of intrathecal OXT subgroup relative to their pre-values and the control group value. The pain threshold was higher in the intrathecal OXT subgroup than that recorded in IV subgroup ($p < 0.001$). However, there was a non-significant ($p > 0.05$) change in the pain threshold after intravenous OXT injection in subgroup (B) when compared with any group. On the other hand, in the OXT group, the pain threshold reduced partially after blocking the analgesic effect of intrathecal OXT by L-glutamate sodium (subgroup E) (Figure 1).

Effect of administration of the analgesic measure on the cardiovascular changes in different studied group

In the experimental study, IV (OXT) subgroup showed a

highly significant ($p < 0.001$) decrease in both ABP and HR but there were non-significant cardiovascular changes ($p > 0.05$) noticed in subgroup (A) (Figure 2).

Measured biochemical parameters

In the experimental study, measurement of serum OXT and dopamine showed a non-significant ($p > 0.05$) change in all studied groups, while serum serotonin was significantly increase in all subgroups relative to control group value. Regarding the brain homogenate level of OXT was significantly ($p < 0.05, 0.01$) increase in subgroups (B, A) versus control group, respectively. While, the homogenate levels of serotonin and dopamine were significantly ($p < 0.05, 0.01$) increase in subgroups (A) in comparison with the control group respectively

Table 1. Serum and brain tissue homogenate experimental biochemical markers measured in the different studied groups of rats.

Item	Control group (n=6)	OXT group(n=12)		Antagonist group(n=6)
		A (Intrathecal OXT)	B (IV OXT)	E (Combined l-glutamate Na ₊ +OXT)
Serum OXT (ng/ml)	15.80±0.44	16.85±1.37	16.43±0.98	15.43±1.32
Brain Homogenate OXT (ng/ml)	20.05±1.29	22.63±0.40 ^a	20.15±1.36	22.22±1.73 ^a
Serum serotonin (ng/l)	7.07±2.140	13.02±3.72 ^{aaa}	12.80±2.81 ^a	6.250±2.48 ^{bbb}
Brain homogenate serotonin (ng/l)	30.20±3.22	36.05±2.43 ^a	30.61±1.51 ^{bbb}	31.32±2.75 ^b
Serum dopamine (ng/l)	1±0.35	1.32±0.34	1.06±0.23	0.9±0.36
Brain Homogenate dopamine (ng/l)	3.9±0.65	5.11±0.15 ^a	3.3±0.22 ^{bbb}	2.95±0.05 ^{bbb}

Values represent mean ± SD. OXT = oxytocin. A: Comparison with control group (^a<0.05; ^{aa}<0.01; ^{aaa}<0.001), B: Comparison with intrathecal OXT (^b<0.05; ^{bb}<0.01; ^{bbb}<0.001).

Table 2. Correlations between pain threshold and OXT level with different studied parameters in subgroup (A) of rats.

Item	OXT	Serotonin	Dopamine	ABP	HR
Pain threshold	0.813	826	0.886	-0.901	-0.853
Pearson (r)	0.049	0.043	0.019	0.014	0.031
p-value	*	*	*	*	*
OXT		0.860	0.950		
Pearson (r)	-	0.028	0.004	-	-
p-value		*	*		

ABP, Arterial blood pressure; HR, Heart rate (*=0.05).

(Table 1). Table 2 showed a significant positive correlation observed between the pain threshold and the brain homogenate level of OXT, serotonin and dopamine in subgroup (A) while it showed a negative correlation between pain threshold and the cardiovascular changes in subgroup (A). A significant positive correlation exists between OXT with serotonin and dopamine in subgroup (A).

Histopathological

The immunohistochemical analysis of subgroup (A) revealed moderately increase in chromogranin A expression (particularly in the outer part of the molecular layer under the surface of the pia matter and in the most neurons of spinal cord) in comparison with the control group (Figure 3).

DISCUSSION

Whereas a large body of evidence exists to confirm OXT reproductive roles but its pain modulating effect, mechanism of action and its effect on the cardiovascular system is not clarified. In the present study, we

hypothesized that intrathecal OXT could perform analgesic function. To test our hypothesis, experimental study was included. It was intended to clarify the analgesic role of OXT and its pathophysiological mechanism.

In the present study, the experimental study confirmed the analgesic effect of intrathecal OXT as it caused a significant increase in pain threshold. This goes with Condes-Lara et al. (2009) animal study, which suggested that OXT plays an important role in pain modulation and analgesia. Intrathecal OXT produces analgesia in both normal (Silva et al, 2013), neuropathic rats (Juif and Poisbeau, 2013) and in dogs with back pain (Huffmeijer et al, 2012)

Juif and Poisbeau, (2013) and Ayar et al (2014) described the pathophysiological mechanism of the analgesic effect of OXT. Juif and Poisbeau (2013) described that OXT stimulates the glutamatergic neurons in lamina II, which recruit GABAergic interneurons resulting in the enhancement of GABAergic and glycinergic spontaneous inhibitory transmissions (Ahmet et al, 2014) ending in presynaptic inhibition of C and Aδ fibers (Condes-Lara et al, 2009). This result goes with our finding of the partial blocking effect of pain relieve that recorded in subgroup (E) of rats. This study is the first study, which uses the intrathecal injection of l-glutamate

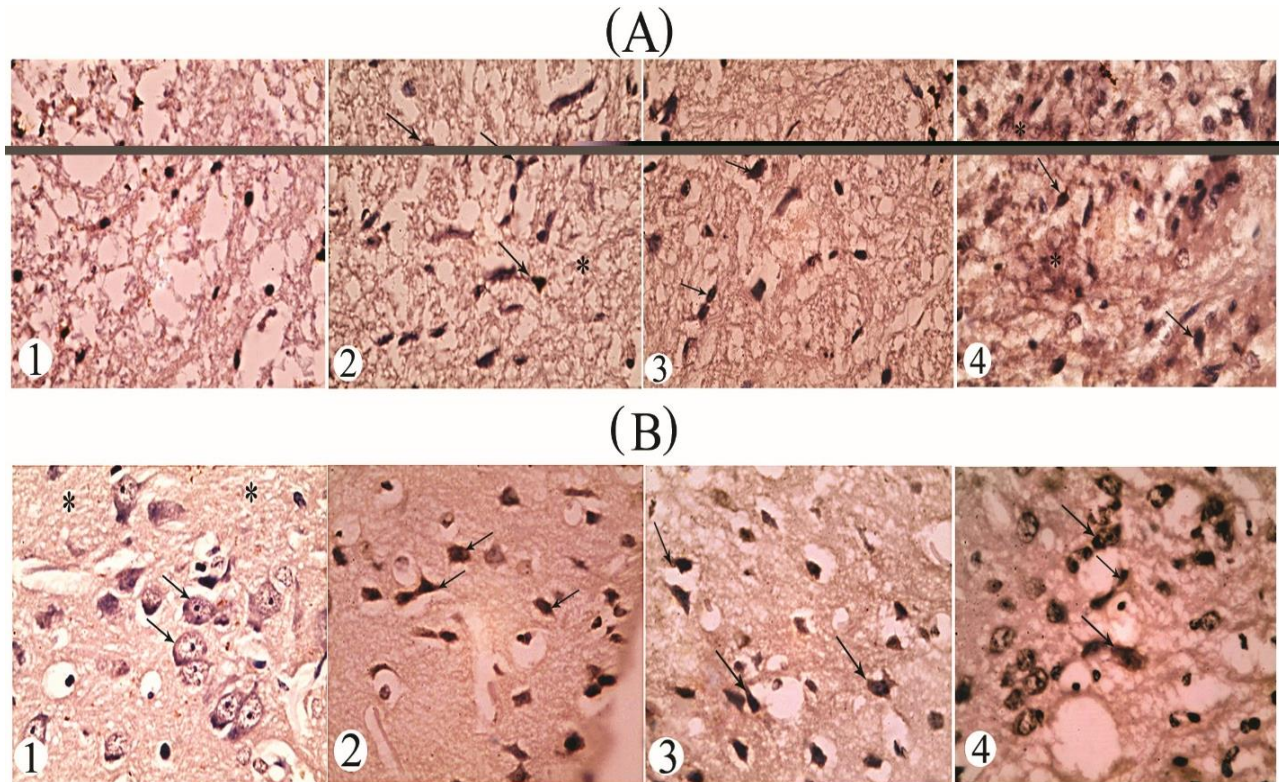


Figure 3. Immunohistochemical changes of the brain (A) and spinal cord(B) tissues in control group (1) and, intrathecal OXT.

sodium as a blocker to intrathecal OXT that indicate that oxytocin may exert some of its analgesic effect through acting on glutamate receptors. Previous studies done by Yang et al. (2011c) found that microinjection of L-glutamate sodium into the SON increases OXT concentrations in the SON perfusion liquid (increase OXT synthesis) and decreases the pain threshold measured by tail flick test.

Moreover, OXT exerts its analgesic effect indirectly through releasing of opiate peptides including leucine-enkephalin, methionine_enkephalin, and beta-endorphin in the periaqueductal gray (Todd, 2010). The present study is the first one proved the relationship between OXT hormone and other neurotransmitters (serotonin and dopamine) in modulating its analgesic action. The significant increase in brain homogenate serotonin level in OXT subgroup (A) of the present study is in agreement with previous animal studies which suggested that serotonergic and oxytocinergic neurotransmission interact anatomically and functionally (Scantamburlo et al, 2009; Montag et al, 2011; Silva et al, 2013). Yaksh et al. (2014) found that rats treated with oxytocin have an increased synthesis of 5-HT in the frontal cortex and brain stem. In addition, brain homogenate dopamine level was significantly increased which coincided with the study of Baskerville and Douglas (2010) who reported increase dopamine release in the ventral striatum and

nucleus accumbens after oxytocin infusion in ventrolateral dopaminergic neurons.

The immunohistochemical examination of OXT subgroup revealed a moderate increase in the expression of chromogranin A immunoreactivity on the molecular layer of the frontal cortex and most of sensory and motor neurons of the spinal cord. This indicates the presence of more oxytocin receptors than control group. This could add another pathophysiological mechanism of the analgesic effect of oxytocin. This goes with Schorscher-Petcu et al. (2010) and Wrobel et al. (2011) studies, which described the presence of specific OXTR located in pain controlling areas in the brain and spinal cord).

It was highly interestingly that intrathecal oxytocin administration showed non-significant changes in arterial blood pressure and heart rate in the experimental animals. This excludes the cardiovascular complications, which goes in line with the previous study of Yang et al. (2011b). However ABP and HR were significantly decreased after intravenous OXT administration and this coincided with the previous study of Yang et al. (2014) which demonstrated that intravenous OXT promoted a direct negative inotropic and chronotropic effects in rats (Yaksh et al., 2014). OXT acts on specific oxytocin atrial cardiomyocytic receptors, stimulate the release of acetylcholine, decreasing the rate and force of cardiac contraction (Montag et al., 2011) and, stimulates the

release of atrial natriuretic peptide (ANP) which causes bradycardia, naturesis and hypotension (Ahmed et al., 2014).

Conclusion

Intrathecal oxytocin is an effective analgesic measure, produce their analgesic effect through direct action on OXTR and stimulation of the release of some neurotransmitters as serotonin, dopamine. In addition, its intrathecal administration can modulate pain concept without major detectable detrimental effects on cardiovascular system.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

- Adrienne R, Wilson P, Edvinas P, Melissa H, Michael MM (2013). The periaqueductal gray contributes to bidirectional enhancement of antinociception between morphine and cannabinoids. *Pharmacol. Biochem. Behav.* 103(3):444-449.
- Ahmed M, Maria F, Donghao W, Bogdan D, Marek J, Jolanta G (2014). Anti-hypertrophic effects of oxytocin in rat ventricular myocytes. *Int. J. Cardiol.* 175(1):38-49.
- Ayar A, Ozcan M, Alcin E, Serhatlioglu I, Ozcan S, Kutlu S, Kelestimur H. (2014) Oxytocin activates calcium signaling in rat sensory neurons through a protein kinase C-dependent mechanism. *J. Physiol. Biochem.* 70(1):43-8.
- Balki S, Mrinalini R, Tsen N, Lawrence M (2014). Oxytocin Protocols for Cesarean Delivery International Anesthesiology Clin. *Int. Anesthesiol. Clin.* 52(2):48-66.
- Baskerville TA, Douglas AJ (2010). Dopamine and oxytocin interactions underlying behaviors: potential contributions to behavioral disorders. *CNS Neurosci. Ther.* 16:e92-e123.
- Condes-Lara M, Rojas-Piloni G, Martinez-Lorenzana G, Lopez-Hidalgo M, Rodríguez-Jiménez J (2009). Hypothalamospinal oxytocinergic antinociception is mediated by GABAergic and opiate neurons that reduce A-delta and C fiber primary afferent excitation of spinal cord cells. *Brain Res.* 1247:38-49.
- da Silva JRT, da Silva ML, Prado WA (2013). Spinal mediation of descending pain inhibitory mechanisms activated by 2/100-Hz electroacupuncture in the rat tail-flick test. *Acupunct. Relat. Ther.* 1(2):15-19.
- Guyton A (2010). *Basic Neuroscience: Anatomy and Physiology*, Elsevier - Health Sciences Division. 1:408. Available at: <https://www.amazon.com/Neuroscience-Anatomy-Physiology-Arthur-Guyton/dp/0721639933>
- Huffmeijer R, Alink LR, Tops M, Grewen KM, Light KC, Bakermans-Kranenburg MJ, Ijzendoorn MH (2012). Salivary levels of oxytocin remain elevated for more than two hours after intranasal oxytocin administration. *Neuro. Endocrinol. Lett.* 33(1):21-25.
- Juif PE, Poisbeau P (2013). Neurohormonal effects of oxytocin and vasopressin receptor agonists on spinal pain processing in male rats. *PAIN* 154(8):1449-1456.
- Montag C, Fiebach C, Kirsch P, Reuter M (2011). Interaction between 5-HT₁PR and a variation on tyeh oxytocin receptor gene influences negative emotionality. *Biol. Psychiatry* 69:601-603.
- Moreno L, Martnez L, Condés L, Rojas P (2013). Identification of oxytocin receptor in the dorsal horn and nociceptive dorsal root ganglion neurons. *Neuropeptides* 47:117-123.
- Scantamburlo G, Ansseau M, Geenen V, Legros J (2009). Oxytocin: from milk ejection to mal-adaptation in stress response and psychiatric disorders. A psychoneuroendocrine perspective. *Ann. Endocrinol.* 70:449-454.
- Schorscher-Petcu A, Sotocinal S, Ciura S, Dupre A, Ritchie J, Sorge RE, Crawley JN, Hu SB, Nishimori K, Young LJ, Tribollet E (2010). Oxytocin-induced analgesia and scratching are mediated by the vasopressin-1A receptor in the mouse. *J. Neurosci.* 30:8274-8284.
- Todd A (2010). Neuronal circuitry for pain processing in the dorsal horn. *Nat. Rev. Neurosci.* 11:823-836.
- Wrobel L, Schorscher-Petcu A, Dupré A, Yoshida M, Nishimori K, Tribollet E (2011). Distribution and identity of neurons expressing the oxytocin receptor in the mouse spinal cord. *Neurosci. Lett.* 495:49-54.
- Yaksh TL, Hobo S, Peters C, Osborn KG, Richter PJ Jr, Rossi SS, Grafe MR, Eisenach JC (2014). Preclinical Toxicity Screening of Intrathecal Oxytocin in Rats and Dogs. *Anesthesiology* 120(4):951-61.
- Yang J, Liang JY, Li P, Pan YJ, Qiu PY, Zhang J, Hao F, Wang DX (2011b). Oxytocin in the periaqueductal gray participates in pain modulation in the rat by influencing the endogenous opiate peptides. *Peptides* 32(6):1255-1261.
- Yang J, Liang JY, Zhang XY, Qiu PY, Pan YJ, Li P, Zhang J, Hao F, Wang DX, Yan FL (2011c). Oxytocin, but not arginine vasopressin is involving in the antinociceptive role of hypothalamic suproptic nucleus. *Peptides* 32(5):1042-1046.
- Yang J, Yu Y, Jian M, Wen Y, Lin BC (2007). Effect of oxytocin on acupuncture analgesia in the rat. *Neuropeptides* 41(5):285-292.
- Yang Y, Wen L, Cao Y, Bao L (2006). Through central arginine vasopressin, not oxytocin and endogenous opiate peptides, glutamate sodium induces hypothalamic paraventricular nucleus enhancing acupuncture analgesia in the rat. *Neurosci. Res.* 54(1):49-56.