Oxytocin Antioxidant Effects on Wistar Rats

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There are very few studies regarding the influence of oxytocin on the oxidative stress status modifications. Even more, the very few studies that exist in this area of research are suggesting controversial results, with reports stating antioxidant, pro-oxidant or sometimes no modifications at all for the specific oxidative stress determined. In this context, in the current study we decided to preliminary study the relevance of 10 days intraperitoneally oxytocin administration on one antioxidant enzyme (glutathione peroxidase-GPX) and one lipid peroxidation marker (malondialdehyde-MDA) in Wistar rats total serum, as compared to a control group of rats which received saline. Thus, we report here a significant antioxidant activity for the administration of oxytocin, as demonstrated by a significant decrease of the specific enzymatic activity of GPX and an increase of MDA concentration in the serum of Wistar rats. This could be relevant in the context of the increased awareness regarding the relevance of oxytocin in the treatment of the main neuropsychiatric disorders, especially when administrated through the intranasal route, considering also that oxidative stress represent an important landmark in these deficiencies.

Key-words: oxytocin, oxidative stress, glutathione peroxidase, malondiadehyde, serum

It is lately believed that besides its well known functions in lactation and labour [1], oxytocin could be extremely relevant in the treatment of the main neuropsychiatric disorders, especially when administrated through the intranasal route [2-6].

Also, it is also well known that oxidative stress (e.g. the alteration of the balance between antioxidant and pro-oxidants) could exert fundamental roles in the pathological manifestation of the neuropsychiatric disorders, as our group also previously demonstrated, in both animal models and human patients with Alzheimer's disease, Parkinson's disease, anxiety, depression, schizophrenia or autism [7-12].

In this context, it could be perhaps relevant to investigate the role that oxytocin could exert on the oxidative stress status. Still, there are very few studies regarding the influence that the administration of oxytocin could exert on the oxidative stress status modifications.

Even more, the very few studies that exist in this area of research are suggesting controversial results, with reports stating antioxidant [13-18], pro-oxidant [19, 20] or sometimes no modifications at all for the specific oxidative stress determined [21].

In this context, in the current study we decided to preliminary study the relevance of 10 days intraperitoneally oxytocin administration on one antioxidant enzyme (glutathione peroxidase-GPX) and one lipid peroxidation marker (malondialdehyde-MDA) in simple Wistar rats total serum, as compared to a control group of rats which received saline.

Experimental part

Material and methods

Male Wistar (n = 14) rats were used. Oxytocin (Pasteur Inst.) was intraperitoneally injected in a dose of 10 mg/kg/ body weight for ten consecutive days. The control rats were also injected with saline.

After collecting the blood, it was allowed to clot and centrifuged immediately. Serum was aliquoted into Eppendorf tubes and stored at -30ºC until measurement.

Biochemical determinations

Determination of glutathione peroxidase

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (SIGMA). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and nicotinamide adenine dinucleotide phosphate (NADPH). The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPX activity [22, 23].

Determination of malondialdehyde

Malondialdehyde (MDA) levels were determined by thiobarbituric acid reactive substances (TBARs) assay. Rat serum (200 µL) was added and briefly mixed with 1 mL of trichloroacetic acid at 50%, 0.9 mL of Tris-HCl (*p*H 7.4) and 1 mL of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100°C for 20 min. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatant was read at 532 nm. The signal was read against an MDA standard curve, and the results were expressed to protein [24, 25].

Data analysis

GPX specific activity and MDA levels were statistically analyzed by using Student's t-test (two tailed, unpaired). All results are expressed as mean ± SEM. $P < 0.05$ was regarded as statistically significant.

Results and discussions

In this way, the administration of oxytocin for ten consecutive days in normal Wistar rats, resulted in a significant increase of the glutathione peroxidase specific activity ($p = 0.037$), when compared to control rats, which received saline (fig. 1).

In addition, the antioxidant effects of oxytocin administration were also confirmed by the significant decrease we observed for the concentration of

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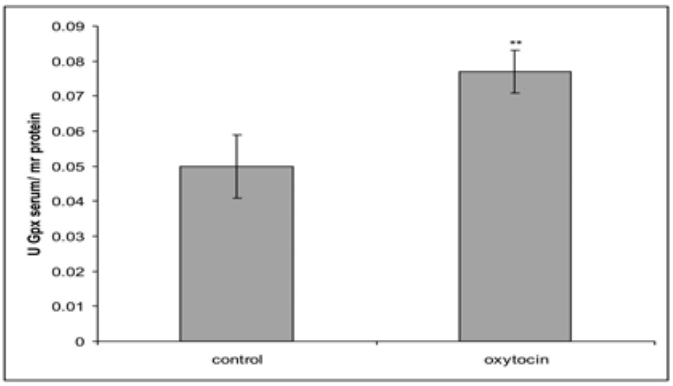

Fig. 1. Glutathione peroxidase specific activity in the serum of rats treated with oxytocin, as compared to controls. The values are expressed as means ± SEM (n = 7 in control, and n = 7 in oxytocin group). **p = 0.037 vs. control group.

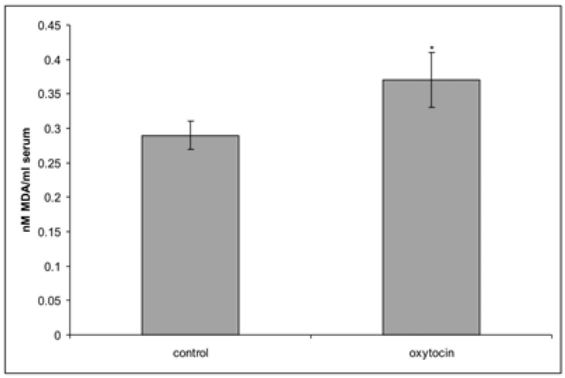

Fig. 2. Malondialhyde concentration in the in serum of rats treated with oxytocin, as compared to controls. The values are expressed as means ± SEM (n = 7 in control, and n = 7 in oxytocin group). **p = 0.043 vs. control group

malondiadehyde (p = 0.043), as a lipid peroxidation marker, in the serum of the rats which received oxytocin, as compared to the controls (fig. 2).

In the present study we report a significant antioxidant activity for a 10 days intraperitoneal administration of oxytocin in the serum of Wistar rats, as demonstrated by a significant decrease of the specific enzymatic activity of GPX and an increase of MDA concentration, as a main lipid peroxidation marker.

Regarding the previous other studies on the oxidative stress status modification as a result of oxytocin administration, the results are quite controversial, with studies on different regions and organs, stating both antioxidant [13-18] and pro-oxidant actions [19, 20].

In this way, the research group of Biyikli et al. [13] showed clear protective effects of oxytocin administration against the oxidative stress induced in a pyelonephritic-rat model, through the administration of E. coli, both acute and chronic. This was demonstrated through the restoration processes for a variety of oxidative stress makers such as malondialdehyde, glutathione and myeloperoxidase, combined with other inflammatory markers, on which we are going to insist later, in the context of the strong connections that might exist between oxytocin and inflammation processes. Thus, the aforementioned authors demonstrated for example that the restorative effect of oxytocin administration on renal glutathione concentration lasted around 7 days [13].

Also, related to inflammatory processes, it was previously demonstrated that oxytocin could be related to cytokine production processes, but also to the nitrosative stress markers [26], as it can affect nitric oxide levels of macrophages in sepsis processes [27].

In addition, the research group of Karelia demonstrated in 2011 [14] that oxytocin could actually exert protective effects through its social roles even in cerebral ischemia (as demonstrated in mice with middle cerebral artery occlusion), a condition which is of course related to increased oxidative stress levels [28, 29]. Thus, the aforementioned authors managed to demonstrate that actually the administration of oxytocin and also the social housing (which of course increased oxytocin levels) attenuated infarct size and decreased oxidative stress levels, as a result of the aforementioned surgery technique [14].

Another example for the oxytocin antioxidant actions was demonstrated indirectly in some oxytocin receptor knockdowns of dermal fibroblasts and keratinocytes, which exhibited decreased levels of glutathione and increased levels of reactive oxygen species in atopic dermatitis [15].

Regarding the mecanistical aspects for some of the aforementioned cited antioxidant and inflammatory actions of oxytocin, we could add the classical study of Szeto group [17], which successfully demonstrated that oxytocin administration is decreasing NADPH-dependent superoxide production by TNFá, as well as IL 6 secretion in cultured cells and atherosclerosis-related experimental design.

In addition, there are other authors describing some complex mechanism which are involving intestinal oxytocin, oxidative stress and various other signaling paths, in the context of the modern autism-gut correlations [30-33].

Similar results were also reported in an experimental rat model of colitis, induced with acetic acid, where oxytocin administration restored MDA levels and increased the levels of glutathione, suggesting important antioxidant actions [18].

In addition, oxytocin exerted antioxidant and protective effects in hepatic ischemia induced in female rat through specific occlusions to median and left liver lobes [21]. However in this specific study, only the MDA levels were decreased as a result of oxytocin administration, while no effect was observed on the specific levels of glutathione determined [21].

Also, in another model of renal ischemia Tugpepe et al. [34] showed that oxytocin increased the levels of glutathione, while also decreasing MDA as a lipid peroxidation marker and the total levels of reactive oxygen species, when determined through a chemiluminescent method.

Very promising results were also observed in diabetic patients with necrotic foot lesions by a Russian research group, where oxytocin treatment actually reportingly increased regenerative potential of endotheliocytes and fibroblasts, as well as stimulating DNA-synthesizing processes [35].

Also, in a very recent and interestingly designed study from 2016 by Napierala group [36], the authors showed in rats that tobacco smoke could affect oxytocin secretion and the related plasma oxidative stress makers, especially considering the well known effects of tobacco smoke and nicotine on the oxidative status [37,38]. Thus the aforementioned authors established a correlation between smoking and affected breastfeeding, which could me mediated partially by some oxidative stress responses [36].

Still, as mentioned before, there are also studies stating pro-oxidant actions for oxytocin administration.

In this way, for example the group of Mostafa et al. demonstrated that the seminal plasma oxytocin levels are significantly increased in infertile patients with varicocele, aspects which are correlated with decreased levels of GPX and increased levels of MDA [20].

In addition, another study demonstrating clear pro-oxidant actions of oxytocin, as demonstrated by altered total oxidant status and total antioxidant capacity, decreased paraoxonase-1 and arylesterase enzymes, as well as an significant increase in the homocysteine and ceruloplasmin oxidase activity, however with the experiment being performed for 6 months in lactating buffaloes [19]. In this way, the authors suggested that during lactation, oxytocin administration is correlated with increased oxidative stress status and further on, with poor productive and reproductive potential in these animals [19].

Even more, there are studies even from 1982 published in Science, stating somehow stimulatory effects of oxytocin on free radical production, through the facilitation of lipogenesis processes [39].

In this way, all these different results could be explained perhaps by the different organs and systems studied (as we present in details above), different dosage, different pathways of administration or different methods used for the determination of the oxidative stress markers [40].

In addition, considering that most of the authors are stating that some reported neuroprotective effects of oxytocin could be explained through a combination of antioxidant and anti-inflamatory effects, while there are also very well known correlation between the inflammation processes and the modification of the oxidative stress status [41, 42] in the next section we will try to describe the connections that might exist between oxytocin and the inflammation processes.

In this way, even from 1994, Spangelo and his team found a correlation between an important inflammatory marker, IL6 and oxytocin. Thus, the administration in vitro of 1 Mmol of vasopressin and oxytocin inhibited the pituitary release of IL-6 from neurointermediate pituitary lobe [43].

In addition, in a study done on animal model, an increase in oxytocin and also vasopressin production was reported after administration of interleukin-1 beta, but not in case of interleukin-6 administration. Interestingly, anterior treatment with anti-inflammatory medication such as aspirin, significantly diminished oxytocin release, after IL-1b treatment. Also, this research simulate IL-1b and IL-6 production after a stressful condition that is usually associated with increased of these cytokines and proved an increase of plasma oxytocin [44].

Also, stressful conditions were created in another classical experiment, in order to determine the reaction of oxytocin to stress. After endotoxin administration a significant increase of oxytocin from mean baseline levels of 5 pg/ml to mean peak levels of 168 pg/mL was observed. In addition, when an anti-inflammatory drug (e.g. indomethacin) was administered, the effect was an attenuated release of oxytocin, demonstrating that inflammation is an important regulator of oxytocin release [45].

Cerebral oxytocin showed also a connection with inflammation in a research done by Landgraf et al. [46] on male rats. In this way, the direct administration of IL-1 in the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei during microdialysis resulted in a rapidly increase of oxytocin release, but only in SON oxytocin reached statistical significance (e.g. up to 178%).

As it is known, oxytocin was best investigated in pregnancy [1] and long term research of oxytocin in tissues which are essential in reproductive functions brought the most consistent evidences regarding a connection between oxytocin and the inflammatory process. In this way, studies focusing on pregnancy modifications found that oxytocin may influence some inflammatory processes by activating NF-êB and inflammatory labour-associated genes including IL-8, CCL5, IL-6 and COX-2 in human myometrium and amnion [47].

Also, other authors found an inverse connection between oxytocin and inflammation is in pregnancy context. Thus, inflammatory cytokines, such as IL-1 b or IL-6, which are involved in labor may influence oxytocin receptor gene expression in myometrial cells. This hypothesis was launched by Schmid et al., [48], which reported that both IL-1 b and IL-6 administration produced a significant decrease, in oxytocin receptor messenger, RNA nuclear-factor-IL6 mediated. Moreover, the authors demonstrated that the transcriptional regulation of the human oxytocin receptor gene by IL-1b and IL-6 depends on a specific region, the 21203/2722 region of the oxytocin receptor promoter, which showed binding sites for nuclear-factor-IL6, acute phase response element and NFk B.

Recently, it was also discovered that oxytocin receptors have a larger distribution in the body and importantly in the cardiovascular system and in the endothelial cells [49].

These findings were the base of further research on the role of oxytocin system in various other sites such as brain, heart, vessels and demonstrated also anti-stress and anti-inflammatory properties. In this way, the effects of oxytocin system in inflammation were evaluated in peripheral tissue where oxytocin and its receptor are expressed, including heart and blood vessels. Thus, in the aforementioned oxidative stress-related study published by Szeto group [17], an anti-inflammatory effect of oxytocin was seen in THP-1 macrophages and endothelial cells. Practically, the team incubated cultured endothelial cells and smooth muscle cells in oxytocin at philologically levels, with results indicating a decrease of inflammatory processes, decreasing LPS-stimulated IL-6 secretion from macrophages by 56% and endothelial cells by 26% [17].

In addition, other authors suggested that oxytocin could have anti-inflammatory properties in other sites than the vascular tissue, due to the fact that macrophages circulate throughout the body. In addition, animal models studies showed that when oxytocin is administered, animal are showing less atherosclerotic lesions and significantly less IL-6 levels, suggesting a possible anti-atherosclerotic role of oxytocin, by decreasing the inflammatory state [50].

Thus, considering all these aforementioned effects of oxytocin on oxidative stress and inflammation, including the one which we are describing in the present report, further speculation and studies regarding the potential use of this easily accessible, cheap and clinically feasible agent [21] as a supplement, adjuvant or therapeutical avenue in some disorders (including the neuropsychiatric disorders we mentioned in the Introduction) seems warranted.

Conclusions

In this report we demonstrated a significant antioxidant activity for the administration of oxytocin, as showed by a significant decrease of the specific enzymatic activity of GPX and an increase of MDA concentration in the serum of Wistar rats. This could be important in the context of the increased awareness regarding the relevance of oxytocin in the treatment of the main neuropsychiatric disorders, especially when administrated through the intranasal route,

considering also that oxidative stress represent a fundamental aspect in these deficiencies, as we mentioned above.

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