Neuro
endocrinology

DOI: 10.1159/000480669
Received: 4/4/2017 3:31:11 AM
Accepted: 8/25/2017
Published(online): 8/27/2017

Role of Oxytocin on Prolactin Secretion During Late Pregnancy
Villegas-Gabutti C. Pennacchio G. Vivas L. Jahn G. Soaje M.

ISSN: 0028-3835 (Print), eISSN: 1423-0194 (Online)
http://www.karger.com/NEN
Neuroendocrinology

Role of Oxytocin on Prolactin Secretion During Late Pregnancy

Villegas-Gabutti C[1], Pennacchio GE[1], Vivas L[2], Jahn G[1], Soaje M [1,3]

[1]Laboratorio de Reproducción y Lactancia, IMBECU-CONICET, Casilla de Correo 855, (5500) Mendoza, Argentina. Tel: 54-261-5244156 and Fax: 54 261 5244001.

[2]Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC-CONICET-Universidad Nacional de Córdoba), Córdoba, Argentina

[3]Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo.

Running Title: Oxytocin and prolactin secretion

Keys words: Oxytocin, prolactin, pregnancy, mifepristone, naloxone, rat

Mailing address: msoaje@mendoza-conicet.gob.ar

Abstract

Backgrounds/Aims: During late pregnancy, blockade of progesterone action by mifepristone (Mp) treatment induces a dopaminergic tone fall that enables naloxone (NAL) administration to release pituitary PRL. We determined whether oxytocin, that stimulates PRL secretion acting directly on anterior pituitary lactotrophs, mediates the stimulatory action of Mp and NAL on PRL secretion during late pregnancy. ***Methods:*** On day 19 of pregnancy, circulating and pituitary oxytocin and PRL levels were measured by RIA, 10, 20 and 30 min after NAL (given at 17.30h) in rats pre-treated with Mp (at 08.00h). Pituitary oxytocin receptors (OTR) expression in Mp treated rats was evaluated by RT-PCR. Activation of oxytocin neurons in Mp-NAL treated rats was measured counting double immunoreactive neurons for Fos and oxytocin (Fos-OT-ir) in SON, medial (PaMM) and lateral (PaLM) magnocellular divisions of PVN. ***Results***: Elevated serum oxytocin and decreased pituitary oxytocin were observed 10 min after NAL administration both in vehicle- and Mp-treated rats. This PRL increase was prevented by previous *ip* administration of an OTR antagonist but intracerebroventricular oxytocin administration was ineffective. Mp increased pituitary OTR expression at 18.00h. Only Mp-NAL increased Fos-OT-ir neurons in the PaMM and SON. ***Conclusions***: These findings suggest that PRL secretion induced by Mp-NAL treatment is preceded by oxytocin release. These results together with the activation of hypothalamic oxytocin neurons and the higher expression of pituitary OTR support the hypothesis that, during late pregnancy, oxytocin may act at pituitary level to facilitate PRL secretion if the inhibitory action of progesterone is blocked.

Introduction

Serum prolactin (PRL) levels are the result of a complex balance between the action of dopamine (DA), the main inhibitory factor regulating its synthesis and secretion, and hypothalamic factors with stimulatory effects such as thyrotropin releasing-hormone (TRH), vasoactive intestinal polypeptide (VIP), oxytocin, vasopressin, serotonin, angiotensin II and others [1]. Although important evidence exists, it is still not sufficiently clear if these factors act directly on lactotrophs to induce PRL release and what are their specific mechanisms of action in the different physiological states of the rat. Among the hypothalamic factors mentioned above, oxytocin has been considered a putative regulator of PRL secretion in the female rat [2]. Oxytocin is a nonapeptide synthesized at hypothalamic levels in the paraventricular (PVN) and supraoptic nuclei (SON) and its main roles are the participation in milk ejection and uterine contractions. Most oxytocin neurons project to the neural pituitary lobe [3], where the hormone is released into the circulation, from where it reaches the anterior pituitary [4]. However, some projections have been described to the median eminence and limbic structures [5].

In vivo and *in vitro* experimental data suggest that oxytocin is involved in PRL release acting directly on the anterior pituitary lactotrophs [6-8]. In fact, these cells express oxytocin receptors (OTR) [9] and they increase markedly in pregnant rats [10]. A rise of serum oxytocin levels occurs before the increase of PRL secretion during the suckling stimulus [11, 12] and an increase of oxytocin in portal blood has been described previous to the proestrous PRL surge [13]. Cervical stimulation induces an immediate increase of oxytocin levels [14] that is followed by a rhythmical secretion of PRL [15]. Furthermore, immunoneutralization of oxytocin attenuates the surges of PRL secretion observed during proestrus, lactation and induced by estradiol administration [16, 17]. Pharmacological blockade of OTRs prevents PRL release during proestrus and in different physiological paradigms [18, 19].

In previous reports, we demonstrated that the blockade of the central inhibitory action of progesterone on PRL release by the antiprogesterone mifepristone (Mp) has a permissive effect on PRL release in rats on day 19 of pregnancy. Thus, although Mp alone is not capable of releasing PRL, the administration of the opioid antagonist naloxone (NAL) induces a PRL surge, suggesting an inhibitory-neuromodulatory role of the opioid system at the end of

pregnancy [20, 21]. The elevated circulating progesterone and placental lactogens typical of pregnancy maintain an elevated dopaminergic tone that blocks any stimulus able to induce PRL secretion [22-24]. We have demonstrated that Mp lowers hypothalamic dopaminergic tone, as shown by decreases in TH expression and activity, but that this effect is not sufficient to increase circulating PRL [25], while NAL alone does not modify dopaminergic tone [25]. Taking these results together we may hypothesize that NAL may act stimulating the release of a PRL releasing factor (PRF) that directly stimulates PRL release from the pituitary in the presence of a low dopaminergic tone.

At the end of pregnancy, previous to parturition, oxytocin levels increase progressively in the neurohypophysis [26]. Interestingly, a central opioid mechanism acts at late pregnancy maintaining the oxytocin neurons inhibited to protect them from stressful situations [27]. In this way, the neurohypophyseal oxytocin store remains intact until the time previous to parturition when oxytocin is necessary [27]. On the last fourth of pregnancy, endogenous opioids acting on mu and kappa opioid receptors inhibit PVN and SON oxytocin neurons blocking its release from the neurohypophysis [27-29] Injections of the opioid antagonist NAL increase immediately oxytocin secretion [26]. Specific mu and kappa opioid agonists prevent the suckling reflex [30] and Fos expression in SON magnocellular neurons [26, 31]. Progesterone also plays an important role within the fine mechanism that regulates oxytocin neurons [32]. At the end of pregnancy, when the fall of progesterone levels is prevented by exogenous progesterone administration, oxytocin neurons are inhibited by other mechanisms independent of opioid action, resulting in a delay in the time of parturition [33]. Thus, the stimulatory effect of NAL on PRL secretion in Mp treated pregnant rats may be exerted through the release of oxytocin that will directly stimulate adenohypophysial PRL liberation.

To test this hypothesis we evaluated: a) the effects of Mp and/or NAL on serum oxytocin levels and pituitary oxytocin content, b) whether the blockade of serum oxytocin levels prevents the PRL secretion induced by Mp and NAL administration and if intracerebroventricular administration of oxytocin induces PRL secretion in Mp treated rats, c) the effect of Mp on the expression of pituitary OTRs and d) whether Mp and/or NAL treatments modify the activation of oxytocin neurons by analyzing the number of double immunoreactive neurons for Fos and oxytocin (Fos-OT-ir) in the supraoptic nucleus (SON) and the medial (PaMM) and lateral (PaLM) magnocellular divisions of the paraventricular nucleus (PVN).

2. Materials and methods

2.1. Animals

Virgin female rats, 3-4 months old (200-220 g), bred in our laboratory and originally of the Wistar strain were used. They were kept in a light (06.00-20.00 h) and temperature (22±2 ºC)-controlled room; rat chow (Cargill, Argentina) and tap water were available ad libitum. Vaginal smears were taken daily; virgin rats showing two or three consecutive 4 day cycles were used. Rats were made pregnant by being caged individually with a fertile male on the night of pro-oestrus. Vaginal smears were checked for the presence of spermatozoa on the following morning and that day was considered day 0 of pregnancy. Rats from our colony normally deliver on day 22 of pregnancy. Animal maintenance and handling were conducted according to the NIH guide for the Care and Use of Laboratory Animals (NIH publication Nº 86-23, revised 1985 and 1991) and the UK requirements for ethics of animal experimentation (Animals Scientific Procedures, Act 1986). All experimental procedures were approved by the Care and Use of Laboratory Animals Committee (CICUAL) of the Faculty of Medical Sciences, National University of Cuyo, Mendoza, Argentina.

2.2 Drugs

Naloxone (NAL), oxytocin and mifepristone (Mp; RU-486; 17β-hydroxy-11β-[4-dimethyl-amino-phenyl]-17α-propinyl-estra-4, 9-dien-3-one) were obtained from Sigma Chemical Co, St Louis, MO, USA. Selective OT antagonist (OTA; desGly-NH_2-d$(CH_2)_5$[D-Tyr^2,Thr^4]$OVT^{a,b}$ST-11-61) was a gift of Dr Maurice Manning, Health Science Campus, The University of Toledo, Ohio, USA.

2.3 Experimental procedures

Experiment 1: This experiment was designed to correlate serum PRL levels after Mp and/or NAL administration with serum oxytocin concentrations and pituitary oxytocin content. Additionally, OTR expression in the anterior pituitaries was determined in vehicle or Mp treated rats. We used the same schedule as previously described [21, 34]. Briefly, Mp was dissolved in sunflower seed oil and injected *s.c.* at 08.00 h on day 19 of pregnancy at a dose of 5 mg/kg. Control animals were injected with the respective volume of vehicle. NAL was

dissolved in 0.9% (w/v) NaCl and injected *i.p.* at a dose of 2 mg/kg at 17.30 h. Groups of 5-8 rats from each experimental condition were decapitated 10, 20 and 30 min after administration of NAL or saline. Trunk blood was collected and the posterior pituitaries were rapidly removed (from the 10 and 20 min groups) and stored frozen (-70 ºC) until assay for oxytocin content by radioimmunoassay (RIA). The blood samples were allowed to clot at room temperature and serum was separated and stored frozen (-20 ºC) until assayed for PRL and oxytocin by RIA.

To evaluate the expression of OTRs, the anterior pituitaries of groups of 6 oil or Mp-treated rats sacrificed 30 min after saline administration, were dissected and stored frozen (-70 ºC) until the RNA extraction.

Experiment 2: The aim of these experiments was to determine if oxytocin is responsible for the systemic increase of PRL induced by Mp and NAL treatment. First,-we determined the effect of systemic OTA administration or icv injection of oxytocin. Pregnant rats treated with Mp as previously described in experiment 1, received an *i.p.* injection of 500 µg/kg OTA dissolved in saline or saline only, five minutes before NAL (n=8) or saline (n=8) administration (at 17.30 h). Rats were decapitated 30 min after.

Other groups of rats had a stainless-steel guide cannulae surgically implanted in the right lateral ventricle on day 12 of pregnancy, seven days before the experiment. The animals were anesthetized with a combination of xylazine hydrochloride (4 mg/kg) and ketamine hydrochloride (80 mg/kg), injected *i.p.* between 09.00 a.m. and 12.00 a.m. Rats were positioned in a stereotaxic frame and a stainless-steel guide cannula was inserted into the right lateral ventricle (M/L 1.5 mm, A/P-0.4 mm relative to bregma, 4.5 mm relative to dura [35]. Cannulae were fixed to the skull using dental acrylic and sealed until the time of drug injection. On the morning of day 19 of pregnancy, the animals were treated with Mp (5 mg/kg, *s.c.*) or its vehicle (oil) at 08.00 h, anesthetized with a combination of xylazine hydrochloride (4 mg/kg) and ketamine hydrochloride (80 mg/kg) injected *i.p.* between 09.00 a.m. and 12.00 a.m. and with sterile procedures a sterile silastic cannula (inside diameter 0.5 mm, outside diameter 0.94 mm, Dow Corning, Midland, Michigan, U.S.A) was inserted into the jugular vein [35]. The cannula was externalized on the back of the head and fixed to the skin with a suture. The catheter was filled with sterile heparinized 0.9 % saline, 30 units/ml (Liquemine, Roche, Buenos Aires, Argentina) and stoppered. After surgery, the rats were housed in individual cages until the

moment of the experiment. At 16.00 h, the cannula was attached to an extension tubing (polythene, outside diameter 1.0 mm) connected to a syringe filled with sterile heparinised saline (20 units/ml) and the rats were left undisturbed for 90 min. At 17.30 h oxytocin (0.2 µg/µl dissolved in saline) or saline was injected in a volume of 5 µl using a 10 µl Hamilton microsyringe connected to an injection needle that protruded 1 mm beyond the tip of the guide cannula placed in the lateral ventricle. Blood samples were taken from the jugular vein 20, 40 and 60 minutes after from groups of 6-8 rats of each experimental condition. After the experiment, the animals were decapitated and the cannulae placement was verified histologically and only those animals that had the cannulae correctly placed were considered.

Experiment 3: The purpose of this experiment was to analyze the activation of oxytocin neurons as shown by double Fos and OT immunohistochemistry in the PVN and SON after Mp and/or NAL treatment. Pregnant rats were treated with Mp or oil followed by NAL or saline as described in experiment 1. Ninety minutes after NAL or saline administration the 4 animals of each experimental condition were perfused for immunohistochemical detection of Fos and oxytocin.

2.4 Hormone determinations

Serum concentration of PRL was measured by double-antibody RIA with materials supplied by Dr A. F. Parlow from the National Hormone and Pituitary Program. PRL was radioiodinated using the chloramine T method and purified by passage through a Sephadex G-75 column. The assay sensitivity was 1 ng/ml serum and the inter- and intra-assay coefficients of variation were less than 10%. The PRL antibody does not cross-react with placental lactogen [36].

Oxytocin was measured by double antibody RIA using an antibody generously provided by Dr N Hagino as previously reported [37]. The hormone was radioiodinated using the chloramine-T method and purified by passage through a Sephadex G50 column. To maximize sensitivity of the assay, the standards and serum samples were incubated 24 h at 4 °C with appropriate dilution of the antibody, subsequently the labelled hormone ($8–10\times10^3$ cpm) was added and the tubes incubated overnight at 4 °C before addition of the second antibody. Assay

sensitivity was 8 pg/ml serum and the intra-assay coefficients of variation were <10%. All the samples were measured on the same assay by duplicate.

2.5 Total RNA Extraction and reverse transcriptase PCR

Total RNA was isolated from anterior pituitaries obtained after Mp or vehicle treatment with the single-step method based on guanidine isothiocyanate/phenol/chloroform extraction using TriZol (GIBCO-BRL, Inc) according to the manufacturer's instructions. RNA concentration was determined by absorbance at 260 nm and its integrity was verified by electrophoresis on 1.5 % agarose gel. Reverse transcription (RT) was carried out using 5 µg of total RNA obtained from the hypophysis of each rat. RT was performed at 37°C for 60 min with 200 U of Moloney murine leukaemia virus reverse transcriptase (GIBCO-BRL, Inc). Before proceeding with the semiquantitative PCR, the conditions were established for each mRNA such that the amplification of the products was in the exponential phase, and the assay was linear with respect to the amount of input cDNA. For the PCR amplification, specific oligonucleotide primers (0.5 µM each) were incubated with aliquots of cDNA template corresponding to 50 ng total RNA in a 35 µl PCR reaction mixture containing 1.5 mM $MgCl_2$, 25 mM KCl, 10 mM Tris-HCl, pH 9, 1µl deoxynucleotides (1mM each), and 1 unit Taq polymerase (Invitrogen Life Sci, Argentina). The sequences of the specific primers for amplification of the OTR mRNA were: Sense: 5′- GCATGTTCGCCTCCACCT-3′, Antisense: 5′-CCTGTGAAGAGCATGTAGATCC-3′ and for amplification of β-actin were: sense 5′-CGTGGGCCGCCCTAGGCACCA-3 and antisense 5′-TTGGCCTTAGGGTTCAGAGGGG-3′ (BC063166). The thermal cycling program for OTR PCR amplification was as follows: 95 °C for 80 sec, 62 °C for 80 sec, 72 °C for 90 sec for 30 cycles, followed by an elongation step of 5 min at 72 °C. The same protocol was used for β-actin amplification with an annealing temperature of 56 °C. Samples from control (n= 8) and treated rats (n= 8) were run and processed simultaneously. RNA samples were assayed for DNA contamination by PCR without the prior reverse transcription. The amplicons (OTR 634 and β-actin 243 bp) were analyzed on 1.5% agarose gels containing 0.5 mg/ml ethidium bromide and photographed with a Kodak DC-290 camera. Band intensities of the RT-PCR products were quantified using the NIH Image software; relative levels of mRNA were expressed as the ratio of signal intensity for the target gene relative to β-actin cDNA.

2.6 Immunohistochemistry

Rats were anesthetized with an *i.p.* injection of chloral hydrate and perfused transcardially with ~200 ml saline followed by 400 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.2). The brains were removed, fixed in the same solution overnight, and then stored at 4 °C in PB containing 30% sucrose. The brains were serially sectioned (40 µm slides) with a cryostat (Microm) beginning approximately at -0.60 mm posterior to bregma, corresponding to the supraoptic nucleus and through the paraventricular nucleus (-0.96 mm to -1.92 mm posterior to bregma) according to the atlas of Paxinos & Watson (2007) for the adult rat brain. Immediately before immunostaining, sections were placed in a mixture of 3% H_2O_2 and 10% methanol until oxygen bubbles ceased appearing and then incubated in 10% normal horse serum (NHS) in PB for 1 h to block sites of nonspecific binding of serum products. Fos-immunoreactivity (Fos-ir) was detected using the standard avidin-biotin peroxidase protocol. The free-floating sections were incubated overnight at room temperature with anti-Fos antibody, raised in rabbits against a synthetic 14-amino acid sequence corresponding to residues 4–17 of human Fos (Ab-5, batch no. 60950101; Oncogene Science, Manhasset, NY) diluted 1:30000 in a solution of PB containing 2% NHS and 0.3% Triton X-100. After washes in PB, sections were subsequently incubated with biotin-labeled anti-rabbit immunoglobulin and ExtrAvidin peroxidase complex (Sigma, 1:20 dilution in 2% NHS-PB) for 1 h at room temperature. The peroxidase label was detected using diaminobenzidine hydrochloride (DAB, Sigma) intensified with 0.5% cobalt chloride and 0.5% nickel ammonium sulphate. This method produces a blue-black nuclear reaction product. One series of Fos-labeled sections was processed subsequently for immunocytochemical localization of oxytocin. Sections were incubated for 72 h at 4 °C with polyclonal rabbit anti-oxytocin antibody (Peninsula Laboratories) and revealed with avidin biotin peroxidase. After incubation, sections were rinsed and incubated with the appropriate biotinylated secondary antiserum and ExtrAvidin peroxidase complex (Sigma, 1:20 dilution in 2% NHS-PB). Cytoplasmic oxytocin immunoreactivity was detected with unintensified DAB to produce a brown reaction product. Finally, the free-floating sections were mounted on gelatin-coated slides, dehydrated and cover slipped with Canada balsam. To check for nonspecific labelling, the primary antibody was omitted in the course of immunostaining. The brain nuclei exhibiting positive staining were identified and delimited according to the rat brain atlas of Paxinos & Watson (2007).

Representative sections exhibiting Fos-oxytocin-ir were identified and delimited according to the atlas of Paxinos & Watson (2007) for the adult rat brain. Double labelled Fos-oxytocin-ir neurons were counted in the supraoptic nucleus (SON) and in two levels for different PVN subnuclei, i.e., medial magnocellular (PaMM) and lateral magnocellular (PaLM). Sections from each brain were obtained based on planes comparable to plates 44, 45 and 47 of the atlas of Paxinos & Watson (2007). The Fos-oxytocin- ir neurons of all nuclei/subnuclei were counted at one level of representative sections in control and experimental groups acquired at exactly the same level. The distance from the bregma of the corresponding plates is as follows: for SON= −1,32 mm; PaMM= −1,44 mm; PaLM= −1,72 mm. The counting was done in four animals of each condition and was repeated at least twice on each section analyzed, to ensure that the number of profiles obtained was similar. The counting was done in four animals of each condition and was repeated at least twice on each section analyzed, to ensure that the number of profiles obtained was similar. Images were taken with a Nikon Eclipse E200 Microscope (Nikon Corp., Japan) fitted with a Micrometric SE Premium digital still camera (Accu-Scope, Commak, NY 11725) under 10× and 100× magnifications. Images were assembled into figures for publication using Adobe Photoshop with minimal alteration to the contrast and background. Figures show representative photomicrographs of SON, PaMM and PaLM sections with double immunoreactive cells for -Fos and oxytocin.

2.7 Statistics

Statistical analysis was performed using two-way analysis of variance (ANOVA) (Figures 1, 2, 4) and Student´s test (Figure 3) with the statistical computer analysis system GraphPad Prism. When ANOVA revealed statistical differences, we applied the Bonferroni post hoc analysis. When variances were not homogeneous log transformation of the data was performed. Differences between means were considered significant at the $p< 0.05$ level.

3. Results

Serum levels of PRL and oxytocin and pituitary oxytocin content in Mp and/or NAL treated rats on day 19 of pregnancy.

As was previously demonstrated [20, 25], serum PRL levels significantly increased 30 min after NAL administration in Mp-treated rats (Fig. 1.A). Interestingly, 20 min after NAL administration, a slight but not significant increase on serum PRL was observed. No effect was obtained after saline administration in vehicle or Mp (Fig. 1.A) treated rats at all times studied. However, increases in serum oxytocin levels were observed 10 min after NAL administration both in vehicle and Mp treated rats (Fig. 1.B). In correlation with serum oxytocin increase, a fall of pituitary oxytocin content was observed 10 min after NAL administration, that was maintained until at least 20 min after, in vehicle and in Mp treated rats (Fig. 1.C), while no effect on oxytocin pituitary content was observed after saline treatment (Fig. 1.C). Since no change was observed on serum oxytocin levels 20 and 30 min after NAL administration in spite of the increases in serum PRL levels, the neurohypophyseal oxytocin content 30 min after NAL injection was not determined.

Effect of oxytocin antagonist (OTA) on serum PRL secretion induced by Mp and/or NAL treatment in rats on day 19 of pregnancy.

To further investigate whether oxytocin mediates the effect of NAL on PRL release in Mp treated rats, we administered OTA to Mp treated rats, 5 min before NAL injection. As shown in Fig. 2, OTA administration prevented the increase on serum PRL secretion induced by NAL in Mp treated rats, but had no significant effect on Mp plus saline treated rats.

Serum PRL levels after icv administration of oxytocin in Mp treated rats on day 19 of pregnancy.

In an attempt to determine whether oxytocin administration can stimulate PRL secretion in Mp treated pregnant rats through central action, oxytocin (0.2 μg/μl) was injected *icv* to vehicle or

Mp treated rats on day 19 of pregnancy and serum samples obtained every 20 min from the moment of injection up to 60 min after. No significant changes in serum PRL levels were observed at the different times studied (Table 1).

Expression of pituitary OTR in Mp treated rats on 19 day of pregnancy.

The administration of Mp (5 mg/kg, *sc*) at 08.00 h on day 19 of pregnancy increased the expression of pituitary OTR mRNA measured by RT-PCR in rats on day 19 of pregnancy (Fig. 3).

Fos activation in oxytocin neurons of the PVN and SON after Mp and/or NAL treatment.

An increase on Fos expression in SON oxytocin neurons was observed after NAL treatment in Mp treated rats (Fig. 4 and 5 B). In the PVN, we found an increase in the number of Fos-oxytocin ir neurons in the PaMM subnucleus of Mp and NAL treated rats (Fig. 4.B and 5.D). In contrast, in the PaLM division no effect of Mp and/or NAL was observed (Fig 4.C). No effects were observed in vehicle plus saline or NAL and in Mp plus saline treated rats (Fig. 4.A and 5.A).

4. Discussion

Strong evidence supports the proposal that oxytocin may act as a PRF in different reproductive states such as the preovulatory PRL surge and lactation [2]. Here we show that the increase on serum PRL levels induced by NAL in Mp treated rats on day 19 of pregnancy, is preceded by an increase in serum oxytocin levels and a fall in posterior pituitary oxytocin content. Moreover, OTA administration five minutes before NAL, resulted in a blockade of the effect of the latter on serum PRL release. These results strongly suggest that NAL is responsible for the release of oxytocin from the neurohypophysis, reflected by the reduced content in the gland and the increase in circulation that in turn, stimulates PRL secretion from the lactotrophs. Thus, oxytocin acting on its own receptors located at pituitary level [1, 2] may be the PRL releasing factor stimulated when the inhibitory opioid tone is lifted in absence of progesterone action, or at least, one of the factors involved. The lack of effect of the *icv* injection of oxytocin to Mp treated rats may suggest that the stimulatory action of the oxytocin release induced by NAL upon PRL release is exerted at the anterior hypophysis level. In fact, central oxytocin effects are different, since it has been reported to modulate magnocellular neurons activity and neurohypophyseal oxytocin release [38, 39], excite TIDA neurons [40] and suppress circulating PRL levels [41, 42] .

Most probably the action of NAL is exerted at hypothalamic level, since opioids modulate the activity of oxytocin neurons acting directly upon them or through other neuron systems that in turn modulate the former [27-29]. Several studies have pointed out that μ-opioid receptors located in hypothalamic nuclei participate in the regulation of oxytocin release from SON and PVN, since NAL administration increases the activity of oxytocin neurons. In fact, it has been demonstrated that μ-opioid receptor activation [28] is involved in the blockade of noradrenergic excitatory inputs to SON and PVN that modulate oxytocin release [43] provided by noradrenergic neurons mainly located in the tractus solitarius nucleus (NTS; A2 neurons) [44]. This is supported by the fact that the blockade of endogenous opioid action by a specific μ-opioid antagonist induces a fall on noradrenaline levels in PVN and SON [45] and increases *c-fos* expression in SON oxytocin neurons [28]. However, a direct inhibitory opioid influence from arcuate nucleus POMC neurons may be also involved because a projection from these

POMC neurons to the PVN and SON is well-established [46, 47]. On the other hand, NAL is a non selective antagonist that acts on all subtypes of opioid receptors, so it may also act on the κ-opioid receptors located in the axon terminals of the neurohypophysis [48] that participate in the regulation of oxytocin release [49], along with μ-opioid receptors that also act at this level [50, 51].

It is also interesting to highlight that the oxytocin release produced by NAL administration in the absence of Mp did not induce an increase on serum PRL levels. Most probably, this is due to the elevated dopaminergic tone present in late pregnant rats [25] that blocks the action of most PRFs [2, 52, 53]. Thus, the oxytocin released by NAL can stimulate PRL release only when the dopaminergic activity is decreased by the antiprogesterone action of Mp. Concomitant to the increase in circulating oxytocin, we found a rapid fall in its neurohypophyseal content in both groups of NAL treated rats. Most oxytocin is secreted into neurohypophyseal capillaries from the nervous terminals of magnocellular neurons located in PVN and SON to the circulation, from where it may arrive to the adenohypophysis to exert its actions, directly through the short portal vessels [54] or indirectly via the general circulation.

On the other hand, the blockade of progesterone action by Mp alone did not modify either serum oxytocin levels or neurohypophyseal oxytocin content. Although a slight expression of progesterone mRNA receptor (PR) in PVN has been measured [32], PRs were not detectable in SON oxytocin neurons [55, 56]. However, evidence indicates that the increased SON *c-fos* expression observed during parturition, is prevented by progesterone administration [33], while Mp administration increases SON *c-fos* expression, suggesting that progesterone mediates an inhibitory action on oxytocin neurons altering directly or indirectly gene transcription via PRs [33]. When progesterone action was blocked by Mp in rats that had been treated with steroids to simulate the hormonal milieu of pregnancy, PVN oxytocin mRNA levels were increased 48 h later [32]. Since in our present study we evaluated Mp action 10 h after administration, it is probable that any action of the antiprogesterone alone needs more time for inducing an effect on oxytocin secretion. Supporting this conclusion, no effect of Mp alone was observed on the expression of Fos in oxytocin-ir neurons in SON, PaMM and PaLM.

Interestingly, in correlation with the increase in oxytocin serum levels after NAL administration to Mp treated rats, a marked activation of SON and PaMM was evidenced by an increase of the number of double –Fos-oxytocin-ir neurons. The lack of effect in PaLM may be

due to the fact that the predominant neuron type present in this PVN division is vasopressinergic, with few oxytocin neurons [57].Although, NAL given alone was able to induce an increase on oxytocin release, no effect on the activation of oxytocin neurons was observed, as demonstrated by an unchanged number of double labelled neurons observed after NAL administration. This result suggests that NAL´s action may involve oxytocin release from axon terminals of the neurohypophysis rather than an action at central levels, in spite of the desensitization of oxytocin axon terminals to endogenous opioid action that occurs at the end of pregnancy [26] .It has been demonstrated that NAL is able to increase SON *c-fos* expression on day 21 of pregnancy [28]. However, in that work no information is given about the levels of serum progesterone of the animals at the moment of sacrifice. This fact is important considering that the authors described in their experimental animals, that parturition occurred during day 21 of pregnancy (11.00-20.00 h). As progesterone prevents NAL-induced *c-fos* expression in SON in late pregnancy[33], it is probable that the diminution of serum progesterone levels that precedes parturition triggered neuronal activation evidenced by an increase of Fos expression. PRs have not been described in the SON [55] and only a low expression was shown in the PVN [32], so it is probable that PRs expressed in the afferent inputs such as GABA neurons [58] mediate the inhibitory effect of progesterone. Although PRs are not expressed in lactotrophs [59] both isoforms (A and B) are present in other pituitary cells as gonadotrophs and may modulate indirectly lactotroph action.

OTRs are highly expressed in the lactotrophs and their expression increases during late pregnancy [10]. Although these receptors are also present in other pituitary cell types such as somatotrophs and gonadotrophs [8],in late pregnant rats most of the OTR mRNA is expressed in the lactotrophs [10]. Thus, the increase in pituitary OTR mRNA induced by Mp treatment may also occur in this cell type. Our finding that Mp treatment increases pituitary OTR mRNA expression suggests that progesterone may inhibit OTR expression, and that after its decrease or PR blockade, lactotrophs increase the expression of OTRs, facilitating the action of oxytocin as a PRF to induce PRL secretion. However, we cannot exclude effects of Mp on OTR expression on the other pituitary cell types.

We have previously shown that estrogen action is necessary for the stimulatory action of Mp and NAL on PRL secretion during late pregnancy, since administration of the antiestrogen tamoxifen on days 14 and 15 of pregnancy prevented it [21, 36]. Thus, it is possible that

estrogen action participates in the activation of oxytocin neurons and particularly in the increase of oxytocin pituitary receptor mRNA after Mp administration. Although a minority of oxytocin neurons express estrogen receptor β (ER β) [60, 61] in PVN and SON, estrogen promotes oxytocin gene expression [60, 62], probably via a nuclear orphan receptor binding site in the oxytocin gene promoter [63]. Also, estradiol-17β rapidly excites oxytocin neurons when chronic opioid inhibition is removed [64]. Moreover, in the anterior pituitary, estradiol increases OTR mRNA [10] and protein [65] expression in ovariectomized rats. Thus, the increase of OTR expression in the anterior pituitary may be induced by estrogens once the action of progesterone has been blocked by Mp.

In conclusion, we present evidence that PRL secretion induced by NAL in Mp pretreated rats is preceded by an increase in serum oxytocin levels, suggesting that this peptide may mediate the stimulatory effect of NAL in absence of progesterone action, acting at pituitary level to induce PRL release. These data also support the hypothesis that oxytocin may act as a PRF during late pregnancy, when the inhibitory action of progesterone is blocked.

Declaration of interest:

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding:

This work has been supported PIP 2012-2014 00863-CONICET (Consejo Nacional de Investigaciones Científicas y Tecnológicas) and Proyecto 06/J458, SeCTyP, Universidad Nacional de Cuyo. CVG, GEP, LV, GAJ and MS are members of CONICET.

Acknowledgments:

The authors are grateful to Mr. J. Rosales for his skilful technical assistance.

FIGURE LEGENDS

Figure 1. *Serum levels of (A) PRL and (B) oxytocin and (C) pituitary oxytocin content in Mp and/or NAL treated rats on 19 day of pregnancy.*

Mifepristone (Mp; 5 mg/kg) or vehicle (V) was administered at 08.00 h and naloxone (NAL; 2 mg/kg) or saline at 17.30 h on day 19 of pregnancy. Animals were sacrificed 10, 20 and 30 min after NAL. Results are expressed as means ± S.E.M. of groups of 5-10 animals in each experimental group. The numbers of animals of each group is indicated inside the corresponding bar. *** p <0.001; ## p <0.01 compared to the respective basal, 20 and 30 min values; **p <0.01; * p <0.05 compared to the respective basal value. Two way ANOVA (factors: time and treatments) followed by Bonferroni post-hoc test.

Figure 2. *Effect of an oxytocin antagonist (OTA) on serum PRL secretion induced by Mp and/or NAL treatment in rats on day 19 of pregnancy.*

Mifepristone (Mp; 5 mg/kg) or vehicle (V) was administered at 08.00 h and naloxone (NAL; 2 mg/kg) or saline (SAL) at 17.30 h on day 19 of pregnancy. Five minutes before NAL administration, the oxytocin antagonist (OTA, 500 μg/kg) or saline were injected *ip.* Animals were sacrificed at 18.00 h on day 19 of pregnancy. Results are means ± S.E.M. of groups of 8 animals in each experimental group. * p<0.01 compared with all the other respective groups. Two way ANOVA (factors: treatment with OTA and treatment with NAL) followed by Bonferroni test for multiple comparisons.

Figure 3. *Expression of pituitary OTR in Mp treated rats on day 19 of pregnancy.*

Upper panel: RT-PCR representative bands. **Lower panel**: OTR mRNA expression levels relative to β-actin. Mifepristone (Mp; 5 mg/kg) or vehicle (V) was administered at 08.00 h, on day 19 of pregnancy. Animals were sacrificed at 18.00 h on day 19 of pregnancy. Results are means ± S.E.M. of groups of 6 animals in each experimental group. * P < 0.05 compared to V (Student´s test).

Figure 4. *Fos activation in oxytocin neurons of the PVN and SON after Mp and/or NAL treatments.*

Mifepristone (Mp; 5 mg/kg) or vehicle (V) was administered at 08.00 h and naloxone (NAL; 2 mg/kg) or saline at 17.30 h on day 19 of pregnancy. Animals were perfused 90 min after NAL

administration. The bars represent the average number of double-immunolabelled Fos-oxytocin (Fos-OT) cells within the SON and the medial magnocellular (PaMM) and lateral magnocellular (PaLM) divisions of the PVN. Results are means ± S.E.M. of 4 animals in each experimental group. For further details see materials and methods section. * $P < 0.05$ compared with the other groups using two way ANOVA (factors: treatment with Mp and treatment with NAL) followed by Bonferroni test for multiple comparisons.

Figure 5. *Pattern of Fos activation in oxytocinergic neurons of the SON and PVN after Mp and/or NAL treatment.*

Mifepristone (Mp; 5 mg/kg) or vehicle (V) was administered at 08.00 h and naloxone (NAL; 2 mg/kg) or saline at 17.30 h on day 19 of pregnancy. Animals were perfused 90 minutes after NAL administration. A and C are representative images of SON and the medial magnocellular (PaMM) division of the PVN respectively, in vehicle plus saline treated rats (magnification: 10x). B and D are representative images of SON and the medial magnocellular (PaMM) division of the PVN respectively in Mp and NAL treated rats. Inset are higher magnifications (x100) of cells indicated in A, B, C, D. Scale = 100 μm. For further details see materials and methods section.

References

1 Freeman ME, Kanyicska B, Lerant A, Nagy G: Prolactin: Structure, function, and regulation of secretion. Physiol Rev 2000;80:1523-1631.

2 Kennett JE, McKee DT: Oxytocin: An emerging regulator of prolactin secretion in the female rat. J Neuroendocrinol 2012;24:403-412.

3 Zimmerman EA, Nilaver G, Hou-Yu A, Silverman AJ: Vasopressinergic and oxytocinergic pathways in the central nervous system. Fed Proc 1984;43:91-96.

4 Samson WK, Schell DA: Oxytocin and the anterior pituitary gland. Adv Exp Med Biol 1995;395:355-364.

5 Knobloch HS, Charlet A, Hoffmann LC, Eliava M, Khrulev S, Cetin AH, Osten P, Schwarz MK, Seeburg PH, Stoop R, Grinevich V: Evoked axonal oxytocin release in the central amygdala attenuates fear response. Neuron 2012;73:553-566.

6 Chadio SE, Antoni FA: Specific oxytocin agonist stimulates prolactin release but has no effect on inositol phosphate accumulation in isolated rat anterior pituitary cells. J Mol Endocrinol 1993;10:107-114.

7 Egli M, Bertram R, Toporikova N, Sellix MT, Blanco W, Freeman ME: Prolactin secretory rhythm of mated rats induced by a single injection of oxytocin. Am J Physiol Endocrinol Metab 2006;290:E566-572.

8 Gonzalez-Iglesias AE, Fletcher PA, Arias-Cristancho JA, Cristancho-Gordo R, Helena CV, Bertram R, Tabak J: Direct stimulatory effects of oxytocin in female rat gonadotrophs and somatotrophs in vitro: Comparison with lactotrophs. Endocrinology 2015;156:600-612.

9 Chadio SE, Antoni FA: Characterization of oxytocin receptors in rat adenohypophysis using a radioiodinated receptor antagonist peptide. J Endocrinol 1989;122:465-470.

10 Breton C, Pechoux C, Morel G, Zingg HH: Oxytocin receptor messenger ribonucleic acid: Characterization, regulation, and cellular localization in the rat pituitary gland. Endocrinology 1995;136:2928-2936.

11 Grosvenor CE, Shyr SW, Goodman GT, Mena F: Comparison of plasma profiles of oxytocin and prolactin following suckling in the rat. Neuroendocrinology 1986;43:679-685.

12 Wakerley JB, O'Neill DS, ter Haar MB: Relationship between the suckling-induced release of oxytocin and prolactin in the urethane-anaesthetized lactating rat. J Endocrinol 1978;76:493-500.

13 Sarkar DK, Gibbs DM: Cyclic variation of oxytocin in the blood of pituitary portal vessels of rats. Neuroendocrinology 1984;39:481-483.

14 Moos F, Richard P: [level of oxytocin release induced by vaginal dilatation (ferguson reflex) and vagal stimulation (vago-pituitary reflex) in lactating rats (author's transl)]. J Physiol (Paris) 1975;70:307-314.

15 Freeman ME, Sterman JR: Ovarian steroid modulation of prolactin surges in cervically stimulated ovariectomized rats. Endocrinology 1978;102:1915-1920.

16 Samson WK, Lumpkin MD, McCann SM: Evidence for a physiological role for oxytocin in the control of prolactin secretion. Endocrinology 1986;119:554-560.

17 Sarkar DK: Immunoneutralization of oxytocin attenuates preovulatory prolactin secretion during proestrus in the rat. Neuroendocrinology 1988;48:214-216.

18 Johnston CA, Negro-Vilar A: Role of oxytocin on prolactin secretion during proestrus and in different physiological or pharmacological paradigms. Endocrinology 1988;122:341-350.

19 McKee DT, Poletini MO, Bertram R, Freeman ME: Oxytocin action at the lactotroph is required for prolactin surges in cervically stimulated ovariectomized rats. Endocrinology 2007;148:4649-4657.

20 Soaje M, Deis RP: A modulatory role of endogenous opioids on prolactin secretion at the end of pregnancy in the rat. J Endocrinol 1994;140:97-102.

21 Soaje M, Deis RP: Opioidergic regulation of prolactin secretion during pregnancy: Role of ovarian hormones. J Endocrinol 1997;155:99-106.

22 Vermouth NT, Deis RP: Prolactin release induced by prostaglandin f2 in pregnant rats. Nat New Biol 1972;238:248-250.

23 Grattan DR, Averill RL: Effect of ovarian steroids on a nocturnal surge of prolactin secretion that precedes parturition in the rat. Endocrinology 1990;126:1199-1205.

24 Arbogast LA, Voogt JL: Progesterone reverses the estradiol-induced decrease in tyrosine hydroxylase mrna levels in the arcuate nucleus. Neuroendocrinology 1993;58:501-510.

25 Soaje M, Valdez S, Bregonzio C, Penissi A, Deis RP: Dopaminergic mechanisms involved in prolactin release after mifepristone and naloxone treatment during late pregnancy in the rat. Neuroendocrinology 2006;84:58-67.

26 Douglas AJ, Dye S, Leng G, Russell JA, Bicknell RJ: Endogenous opioid regulation of oxytocin secretion through pregnancy in the rat. J Neuroendocrinol 1993;5:307-314.

27 Brunton PJ, Bales J, Russell JA: Allopregnanolone and induction of endogenous opioid inhibition of oxytocin responses to immune stress in pregnant rats. J Neuroendocrinol 2012;24:690-700.

28 Douglas AJ, Neumann I, Meeren HK, Leng G, Johnstone LE, Munro G, Russell JA: Central endogenous opioid inhibition of supraoptic oxytocin neurons in pregnant rats. J Neurosci 1995;15:5049-5057.

29 Brunton PJ, McKay AJ, Ochedalski T, Piastowska A, Rebas E, Lachowicz A, Russell JA: Central opioid inhibition of neuroendocrine stress responses in pregnancy in the rat is induced by the neurosteroid allopregnanolone. J Neurosci 2009;29:6449-6460.

30 Clarke G, Wright DM: A comparison of analgesia and suppression of oxytocin release by opiates. Br J Pharmacol 1984;83:799-806.

31 Luckman SM, Antonijevic I, Leng G, Dye S, Douglas AJ, Russell JA, Bicknell RJ: The maintenance of normal parturition in the rat requires neurohypophysial oxytocin. J Neuroendocrinol 1993;5:7-12.

32 Thomas A, Shughrue PJ, Merchenthaler I, Amico JA: The effects of progesterone on oxytocin mrna levels in the paraventricular nucleus of the female rat can be altered by the administration of diazepam or ru486. J Neuroendocrinol 1999;11:137-144.

33 Antonijevic IA, Russell JA, Bicknell RJ, Leng G, Douglas AJ: Effect of progesterone on the activation of neurones of the supraoptic nucleus during parturition. J Reprod Fertil 2000;120:367-376.

34 Gabutti CV, Ezquer M, Deis R, Maldonado C, Soaje M: Pituitary changes involved in prolactin secretion induced by mifepristone and naloxone during late pregnancy. Neuroendocrinology 2009;89:200-209.

35 Valdez SR, Pennacchio GE, Gamboa DF, de Di Nasso EG, Bregonzio C, Soaje M: Opioid modulation of prolactin secretion induced by stress during late pregnancy. Role of ovarian steroids. Pharmacol Rep 2014;66:386-393.

36 Villegas-Gabutti C, Pennacchio GE, Jahn GA, Soaje M: Role of estradiol in the regulation of prolactin secretion during late pregnancy. Neurochem Res 2016;41:3344-3355.
37 Valdez SR, Penissi AB, Deis RP, Jahn GA: Hormonal profile and reproductive performance in lactation deficient (ofa hr/hr) and normal (sprague-dawley) female rats. Reproduction 2007;133:827-840.
38 Neumann ID, Landgraf R: Balance of brain oxytocin and vasopressin: Implications for anxiety, depression, and social behaviors. Trends Neurosci 2012;35:649-659.
39 Landgraf R, Neumann ID: Vasopressin and oxytocin release within the brain: A dynamic concept of multiple and variable modes of neuropeptide communication. Front Neuroendocrinol 2004;25:150-176.
40 Briffaud V, Williams P, Courty J, Broberger C: Excitation of tuberoinfundibular dopamine neurons by oxytocin: Crosstalk in the control of lactation. J Neurosci 2015;35:4229-4237.
41 Mogg RJ, Samson WK: Interactions of dopaminergic and peptidergic factors in the control of prolactin release. Endocrinology 1990;126:728-735.
42 Yuan ZF, Pan JT: Stimulatory effect of central oxytocin on tuberoinfundibular dopaminergic neuron activity and inhibition on prolactin secretion: Neurochemical and electrophysiological studies. Endocrinology 1996;137:4120-4125.
43 Ji Y, Mei J, Lu S: Opposing effects of intracerebroventricularly injected norepinephrine on oxytocin and vasopressin neurons in the paraventricular nucleus of the rat. Neurosci Lett 1998;244:13-16.
44 Onaka T, Luckman SM, Antonijevic I, Palmer JR, Leng G: Involvement of the noradrenergic afferents from the nucleus tractus solitarii to the supraoptic nucleus in oxytocin release after peripheral cholecystokinin octapeptide in the rat. Neuroscience 1995;66:403-412.
45 Kutlu S, Yilmaz B, Canpolat S, Sandal S, Ozcan M, Kumru S, Kelestimur H: Mu opioid modulation of oxytocin secretion in late pregnant and parturient rats. Involvement of noradrenergic neurotransmission. Neuroendocrinology 2004;79:197-203.
46 Douglas AJ, Bicknell RJ, Leng G, Russell JA, Meddle SL: Beta-endorphin cells in the arcuate nucleus: Projections to the supraoptic nucleus and changes in expression during pregnancy and parturition. J Neuroendocrinol 2002;14:768-777.

47 Kiss JZ, Cassell MD, Palkovits M: Analysis of the acth/beta-end/alpha-msh-immunoreactive afferent input to the hypothalamic paraventricular nucleus of rat. Brain Res 1984;324:91-99.

48 Sumner BE, Douglas AJ, Russell JA: Pregnancy alters the density of opioid binding sites in the supraoptic nucleus and posterior pituitary gland of rats. Neurosci Lett 1992;137:216-220.

49 Herkenham M, Rice KC, Jacobson AE, Rothman RB: Opiate receptors in rat pituitary are confined to the neural lobe and are exclusively kappa. Brain Res 1986;382:365-371.

50 Ortiz-Miranda SI, Dayanithi G, Coccia V, Custer EE, Alphandery S, Mazuc E, Treistman S, Lemos JR: Mu-opioid receptor modulates peptide release from rat neurohypophysial terminals by inhibiting ca(2+) influx. J Neuroendocrinol 2003;15:888-894.

51 Ortiz-Miranda S, Dayanithi G, Custer E, Treistman SN, Lemos JR: Micro-opioid receptor preferentially inhibits oxytocin release from neurohypophysial terminals by blocking r-type ca2+ channels. J Neuroendocrinol 2005;17:583-590.

52 Haisenleder DJ, Moy JA, Gala RR, Lawson DM: The effect of transient dopamine antagonism on thyrotropin-releasing hormone-induced prolactin release in pregnant rats. Endocrinology 1986;119:1980-1988.

53 Martinez de la Escalera G, Weiner RI: Mechanism(s) by which the transient removal of dopamine regulation potentiates the prolactin-releasing action of thyrotropin-releasing hormone. Neuroendocrinology 1988;47:186-193.

54 Kiss A, Mikkelsen JD: Oxytocin--anatomy and functional assignments: A minireview. Endocr Regul 2005;39:97-105.

55 Francis K, Meddle SL, Bishop VR, Russell JA: Progesterone receptor expression in the pregnant and parturient rat hypothalamus and brainstem. Brain Res 2002;927:18-26.

56 Rainbow TC, McGinnis MY, Krey LC, McEwen BS: Nuclear progestin receptors in rat brain and pituitary. Neuroendocrinology 1982;34:426-432.

57 Rhodes CH, Morrell JI, Pfaff DW: Immunohistochemical analysis of magnocellular elements in rat hypothalamus: Distribution and numbers of cells containing neurophysin, oxytocin, and vasopressin. J Comp Neurol 1981;198:45-64.

58 Leigh AJ, Carter ND, Horton R, Silverligh JJ, Wilson CA: Ovarian steroid regulation of glutamic acid decarboxylase gene expression in individual hypothalamic nuclei. J Neuroendocrinol 1990;2:433-438.

59 Fox SR, Harlan RE, Shivers BD, Pfaff DW: Chemical characterization of neuroendocrine targets for progesterone in the female rat brain and pituitary. Neuroendocrinology 1990;51:276-283.

60 Shughrue PJ, Dellovade TL, Merchenthaler I: Estrogen modulates oxytocin gene expression in regions of the rat supraoptic and paraventricular nuclei that contain estrogen receptor-beta. Prog Brain Res 2002;139:15-29.

61 Forsling ML, Kallo I, Hartley DE, Heinze L, Ladek R, Coen CW, File SE: Oestrogen receptor-beta and neurohypophysial hormones: Functional interaction and neuroanatomical localisation. Pharmacol Biochem Behav 2003;76:535-542.

62 Peter J, Burbach H, Adan RA, Tol HH, Verbeeck MA, Axelson JF, Leeuwen FW, Beekman JM, Ab G: Regulation of the rat oxytocin gene by estradiol. J Neuroendocrinol 1990;2:633-639.

63 Koohi MK, Ivell R, Walther N: Transcriptional activation of the oxytocin promoter by oestrogens uses a novel non-classical mechanism of oestrogen receptor action. J Neuroendocrinol 2005;17:197-207.

64 Brown CH, Brunton PJ, Russell JA: Rapid estradiol-17beta modulation of opioid actions on the electrical and secretory activity of rat oxytocin neurons in vivo. Neurochem Res 2008;33:614-623.

65 Tabak J, Gonzalez-Iglesias AE, Toporikova N, Bertram R, Freeman ME: Variations in the response of pituitary lactotrophs to oxytocin during the rat estrous cycle. Endocrinology 2010;151:1806-1813.

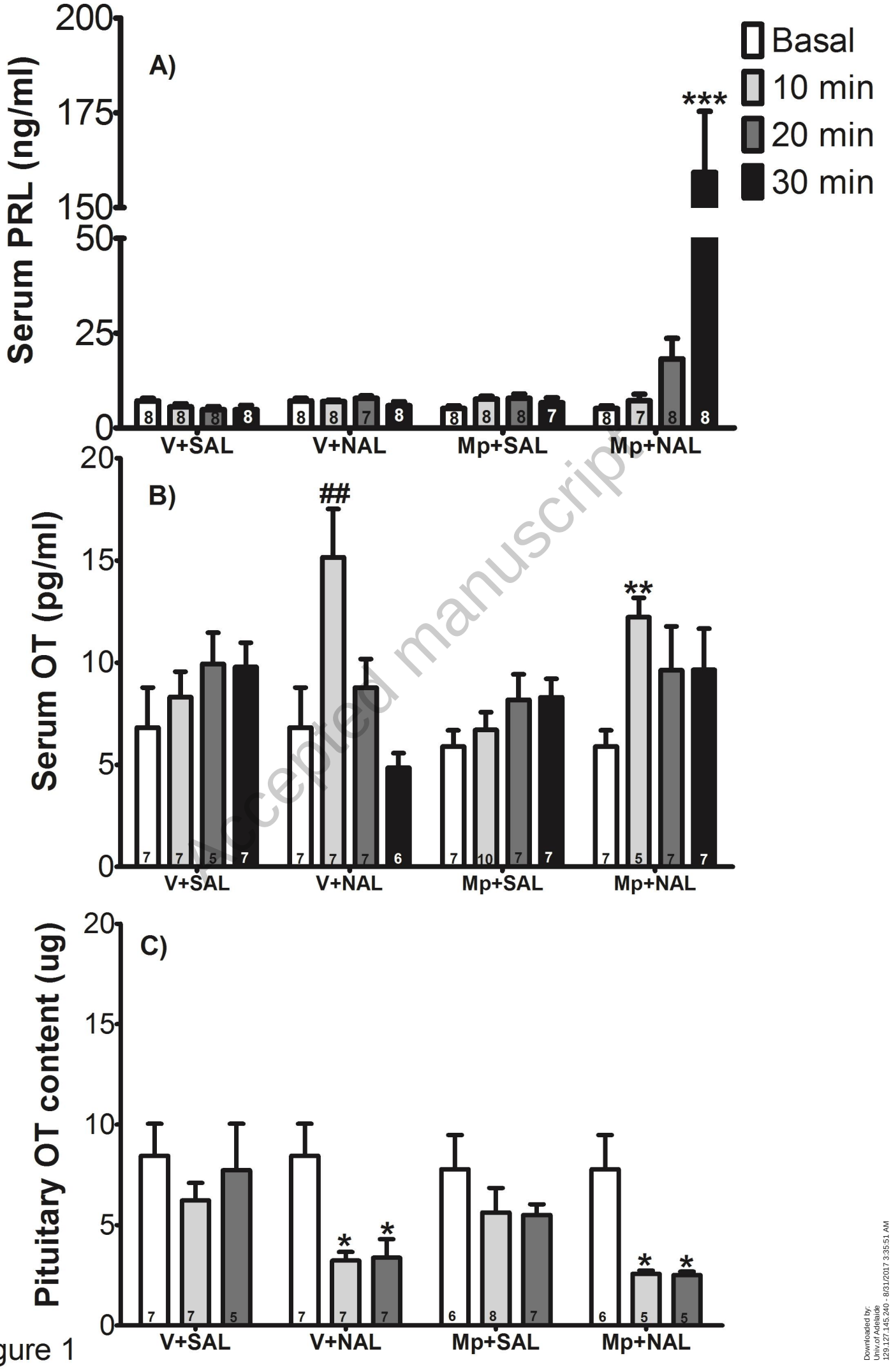
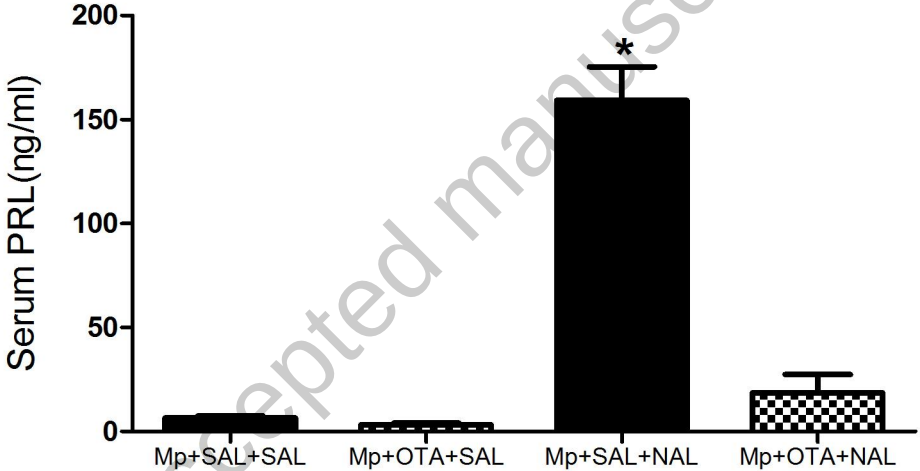


Figure 1



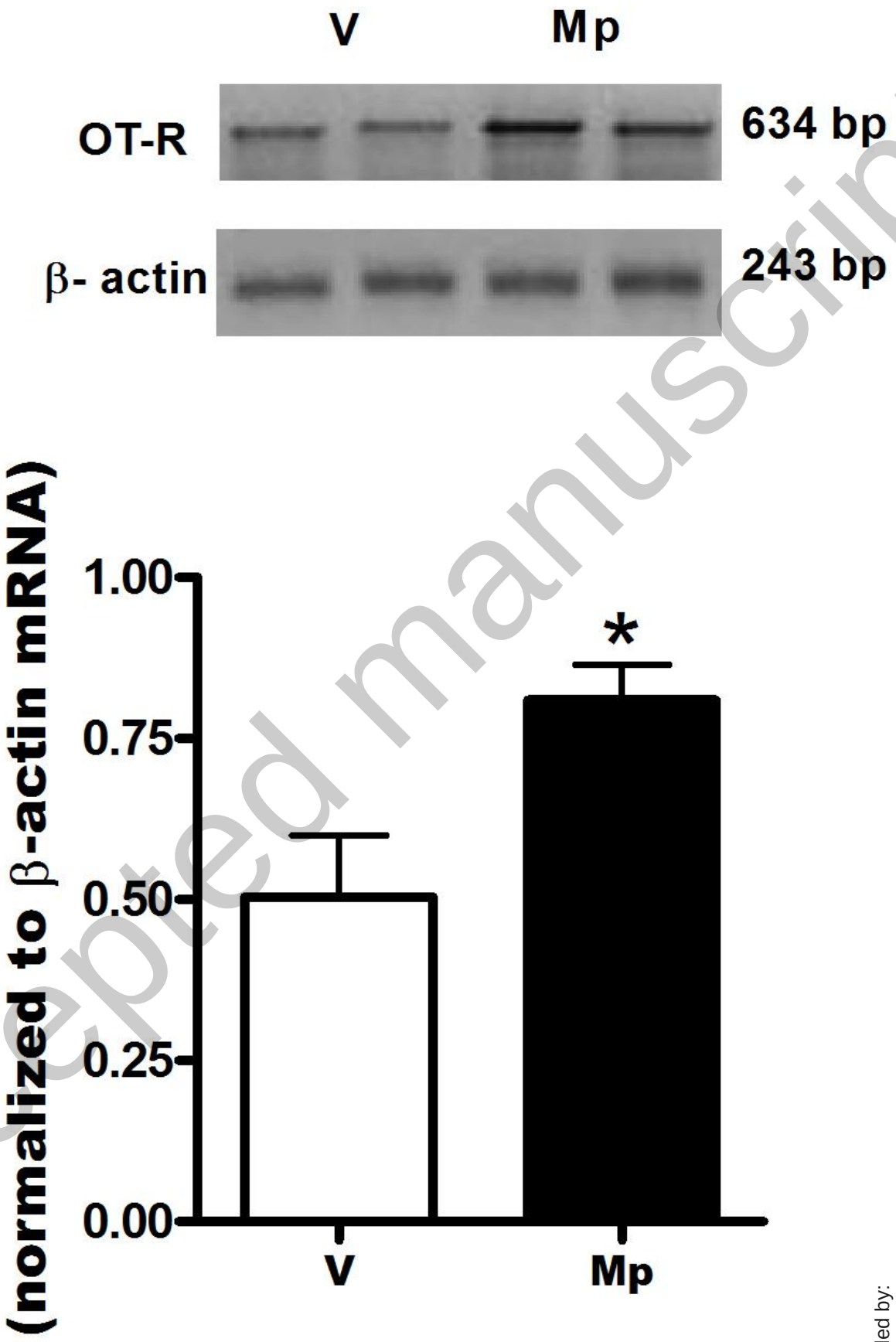


Figure 3

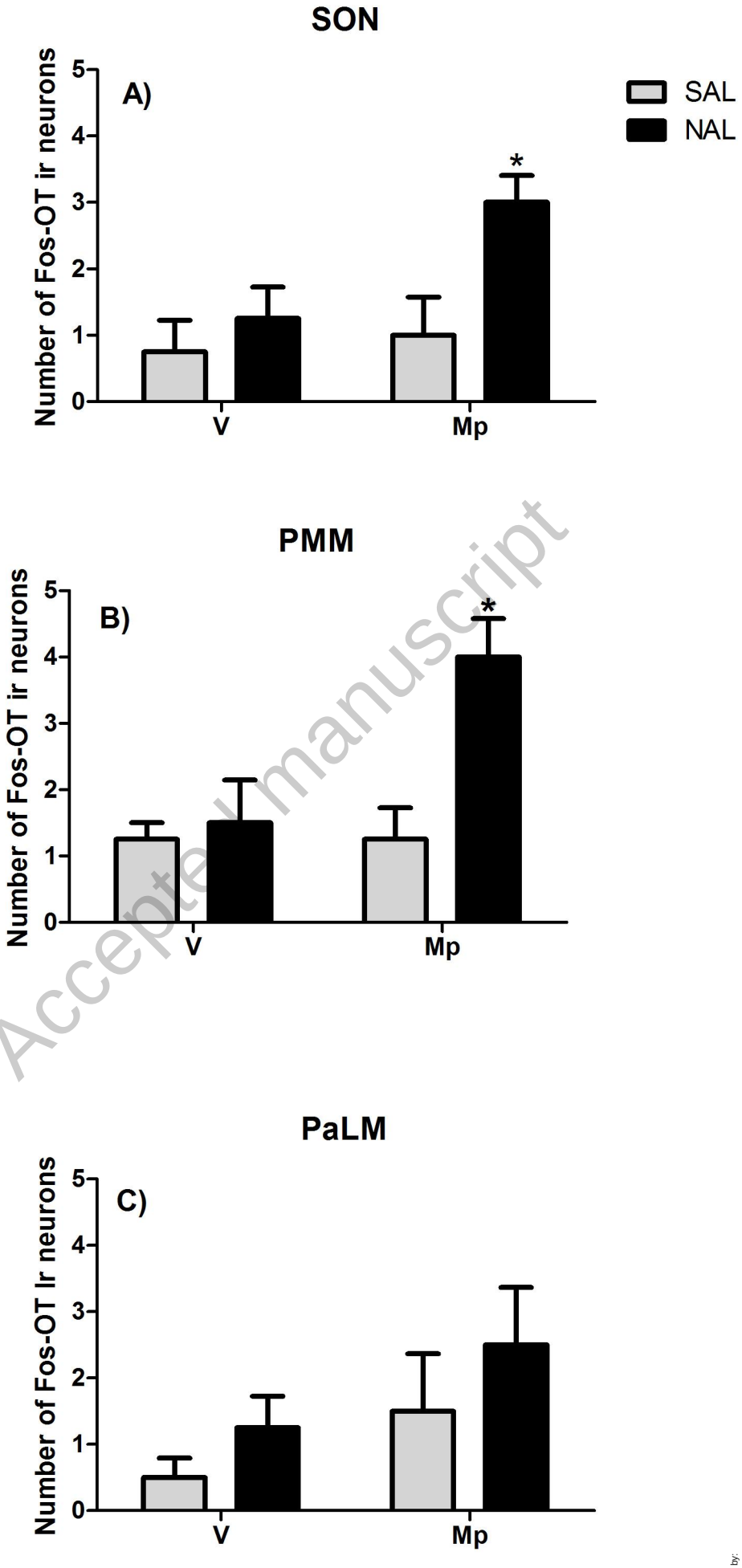


Figure 4

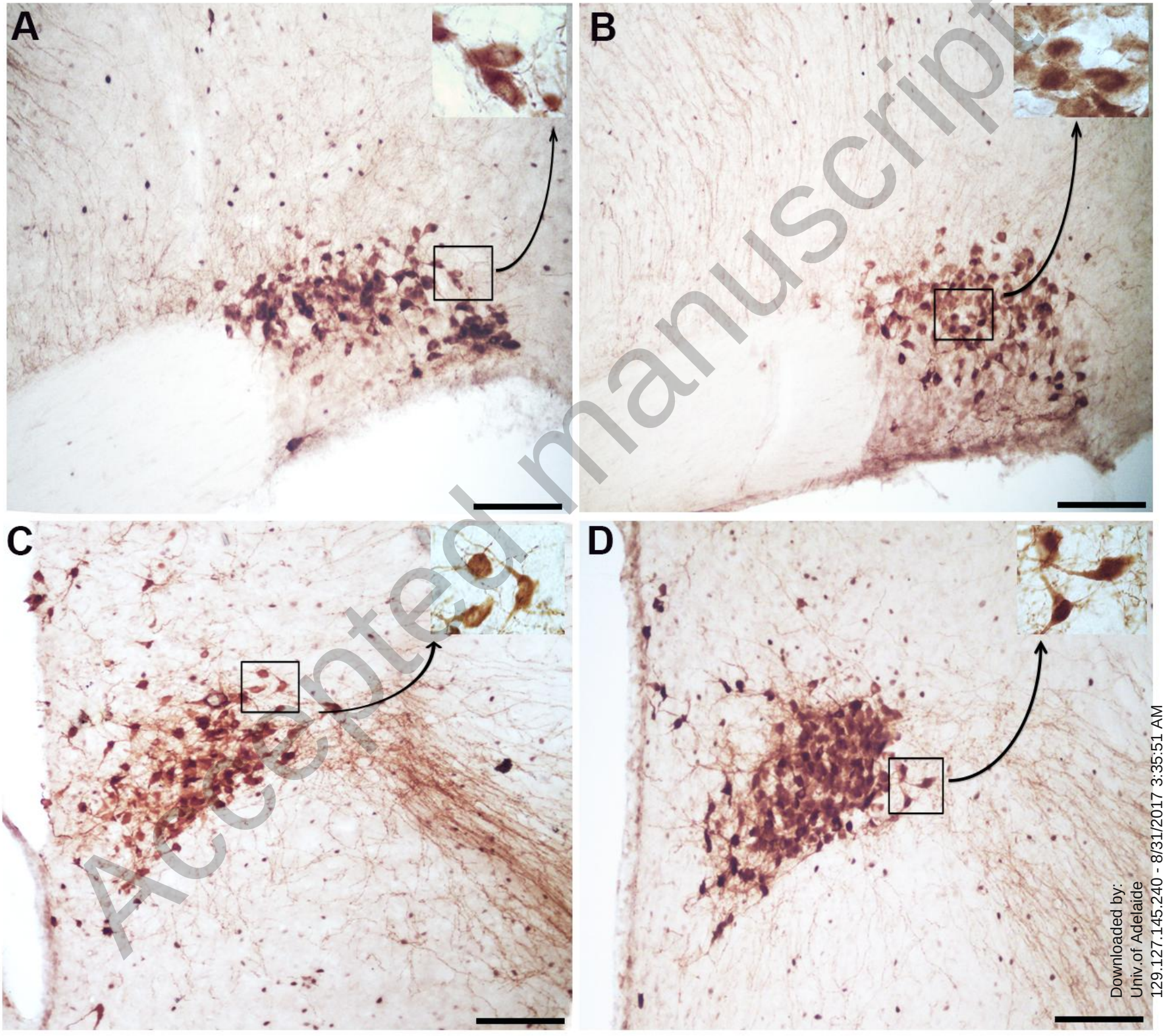


Table 1. *Effect of icv oxytocin injection in mifepristone treated rats on serum prolactin levels on day 19 of pregnancy.*

Serum Prolactin (ng/ml)				
	Oil + saline	Oil + OT	Mp + saline	Mp + OT
Basal	2.8 ± 0.8 (n=6)	2.4 ± 0.5 (n=5)	2.4 ± 0.4 (n=6)	2.2± 0.5 (n=6)
20 min	3.4 ± 0.5 (n=8)	3.0 ± 0.9 (n=7)	2.7± 0.2 (n=9)	1.8 ± 0.2 (n=6)
40 min	2.6 ± 0.5 (n=7)	1.9 ± 0.8 (n=5)	2.6± 0.6 (n=7)	2.2 ± 0.6 (n=7)
60 min	3.8 ± 0.8 (n=7)	2.5 ± 0.5 (n=5)	2.9 ± 0.5 (n=8)	2.9 ± 0.5 (n=6)

Mifepristone (Mp; 5 mg/kg) or vehicle (V) was administered *s.c.* at 08.00 h and oxytocin (OT; 0,2 µg/µl) or saline was injected *icv* at 17.00 Serum samples obtained every 20 min from the moment of injection up to 60 min. after. Results are means ± S.E.M of groups of 5-9 animals in each experimental group.