Allopregnanolone Alters the Luteinizing Hormone, Prolactin, and Progesterone Serum Levels Interfering with the Regression and Apoptosis in Rat Corpus Luteum

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Key words
- ovary
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Abstract

Steroids synthesized in the central nervous system are termed "neurosteroids". They are synthesized and metabolized in several brain areas. The objective of this work was to determine if 1 intracerebroventricular allopregnanolone injection in rats can interfere in luteal regression in a close relationship with modifications in LH, progesterone, and prolactin serum concentrations. Allopregnanolone was injected during proestrus morning and the animals were sacrificed on oestrous morning. Ovulation test and histological analysis were performed in the oestrus morning with light and electron microscopy. Serum prolactin, LH, and progesterone levels were measured by radioimmunoassay. The allopregnanolone injection significantly decreased luteinizing hormone serum level and the number of oocytes on oestrus. Progesterone and prolactin serum levels were increased after this injection. The inhibition of apoptotic figures due to allopregnanolone administration was detected in the already formed corpora lutea belonging to the previous ovary cycle and it was significantly lower than in vehicle group (control). When the $GABA_A$ antagonist (bicuculline) was administered alone or previously to allopregnanolone, no effect on the ovulation rate was observed. No changes in the apoptotic cell numbers were observed with respect to those of vehicle group. These results show that the effect of centrally injected allopreganolone over reproductive function could be due to a centrally originated LH mediated effect over ovarian function that affects luteal regression, through the inhibition of apoptosis and stimulation of progesterone and prolactin release.

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Bibliography
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Introduction

Steroids synthesized in the central nervous system are currently termed "neurosteroids". They are known to be synthesized and metabolized in several brain areas (i.e., cortex, hypothalamus, and hippocampus). The neurosteroids have been reported as important modulators of functions of the central nervous system activities [1]. Changes in neurosteroids levels during development and in adults are involved in many physiological and pathological processes as premenstrual syndrome, stress, depression, epilepsy, and Alzheimer's disease [2]. Neurosteroids affect brain function through actions at their cognate estrogen and progesterone receptors, or modulating receptors whose primary transmitter is not a steroid (e.g., $GABA_A$ receptor) [3].

Particularly, allopregnanolone is a neuroesteroid, which is a positive allosteric modulator of $GABA_A$ receptors exhibiting anxiolytic and anticonvulsant properties [4,5]. It has been shown that these neurosteroids facilitate GABA action at nanomolar concentrations and open the chloride channel at micromolar concentrations [6–10]. The mechanism of action of neurosteroids is known to be involved at 2 different kinds of responses: a fast one (nongenomic), through cell membranes called a surface effect, which modulates ion-channel associated receptors. The slow response (genomic), is mediated by a cytosolic receptor that activates genomic expression of specific proteins [11]. Changes in $GABA_A$ receptor binding affinity after estradiol administration have been attributed to genomic mechanisms whereas the rapid effects of these neurosteroids would occur as a result of a direct interaction with $GABA_A$ receptor [12,13].

A new approach to understand the mechanism of action of neurosteroids derived from Micevych et al. [14], who have demonstrated that estrogen-induced increase of neuroprogesterone precedes

the LH surge, which is blocked by an inhibitor of 3β-HSD, the enzyme that converts pregnenolone to progesterone. These data suggest that de novo synthesis of progesterone from cholesterol in the hypothalamus is a critical part of the estrogen positive-feedback mechanism that stimulates the LH surge. Previously we have reported that 30 min after intracerebroventricular administration of allopregnanolone in ovariectomized (OVX) estrogen-progesterone primed rat inhibits lordosis behavior [15]. The injection of the $GABA_A$ antagonist bicuculline, prior to allopregnanolone, blocked the effects. In the same way, we found an increase in endogenous dopamine concentration with a decreased the turnover rate in medial basal hypothalamus and preoptic area. Moreover, in vitro ^{3}H-dopamine release in medial basal hypothalamus slices was less in allopregnanolone injected rats [15]. On the other hand, the inhibitory effect of allopregnanolone on LH surge has been shown as related to a decrease of dopaminergic activity in the medial basal hypothalamus [15]. In addition, an increase in dopaminergic activity in the preoptic hypothalamic area has been related to an ovulation delay in rats [16], associated with modifications in serum prolactin levels.

We demonstrated that allopregnanolone enhances a modulatory action of NMDA receptors with a direct effect on hypothalamic LHRH release and over the neuronal network afferent to LHRH neurons that are involved in the glutamatergic system [17]. Taken together, these studies showed that the influence of allopregnanolone on dopaminergic, glutamatergic and GABAaergic systems could play an important role in the reproductive function.

During the estrous cycle, the preovulatory surge of LH causes ovulation in the evening of proestrus and the subsequent formation of corpus luteum (CL), consisting in luteinization of follicular cells, endothelial cell invasion, and tissue remodeling. The last one includes changes in the extracellular matrix (ECM) [18,19]. The CL in rodents is considered a transient endocrine gland that forms after ovulation, contributes to the regularity of the estrous cycle and becomes fully functional during pregnancy. Its main secretory product is progesterone (Pg), obtained from pregnenolone by the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD). Dynamic changes in cellular composition and response to different regulators, facilitates the transition between the different reproductive cycles [18]. Luteal regression (luteolysis) is defined as a decrease in the secretion of Pg (functional regression) [20] and subsequent involution of the luteal tissue, including cells with and without steroidogenic characteristics (structural regression) [21]. The structural involution of the corpora lutea is, however, clearly evident only after proestrus stage as a consequence of an apoptotic phenomenon, but little is known about the mechanisms that could mediate the central allopregnanolone actions on the ovarian physiology. We propose that allopregnanolone could act on the corpus luteum maintaining its capacity inhibiting apoptosis even while structural regressing began.

The purpose of the present investigation is to determine whether centrally administered allopregnanolone could interfere with luteal regression process in a close relationship with changes in LH, prolactin and progesterone serum levels. To study the actions of allopregnanolone on the rat ovary, we tested the ovarian morphological and histological changes in close relationship with modifications in neuroendocrine responses.

Materials and Methods

Animals

Adult female rats bred in our laboratory (Sprague-Dawley strain) weighing 200–250 g were used in these experiments. They were housed at a temperature of 22 ± 2 °C and 12-h light schedule (lights on at 07:00 AM) with controlled environment for food and water ad libitum (standard rat chow Cargil, Córdoba, Argentina). They were housed in groups of 4 animals/cage. Animals for experiments were kept and handled according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals of the National Research Council (National Academies, U.S.A., 8th Edition, 2011).

Drugs

Allopregnanolone [3α-hydroxy-5α-pregnan-20-one, (Sigma Chemical Co., St. Louis, MO, USA)], bicuculline methiodide 1(S) B, (Research Biochemical's International Inc, MA, U.S.A), penicillin G benzathine (Riched, Argentina), and chloral hydrate (Anedra, Argentina) were used. Stocks of allopregnanolone were initially dissolved in propyleneglycol to a concentration of 0.6 mM. In order to make negligible the final amount of propyleneglycol, the dose of allopregnanolone used in the experiment (6 µM) was obtained by dilution in Krebs-Ringer bicarbonate glucose (KRBG) buffer at pH 7.4. Not with standing, control animals were injected with KRBG (vehicle) containing propyleneglycol in equivalent concentrations to that used in experimental groups. The dose of allopregnanolone was chosen to mimic its maximal circulating level during stress [22]; and it was used in our previous report of sexual and anxiety behavior effects [4]. Drugs were injected intracerebroventricularly (i.c.v.) in 1 µl injection volume.

Surgical procedures

The stainless-steel cannulae assembly was stereotaxically inserted into the right lateral ventricle in rats under chloral hydrate anesthesia (400 mg/kg i.p.). The following coordinates from bregma were used, in accordance with the coordinates of Paxinos and Watson's Atlas [23], AP: +0.4 mm, L: −1.5 mm, and DV: −4 mm. At the end of the surgery, cannulae were sealed with a stainless steel wire to protect them from obstruction. To prevent infections, each animal received an injection of 0.2 ml intramuscular of 1 200 000 UI penicillin G benzathine (1 UI = 0.6 µg; 72 mg/rat). Animals were housed singly in Plexiglas cages and were maintained for a week undisturbed in order to recover the estrous cycle. At the end of the experiments, the location of the guide cannula into the lateral ventricle was confirmed by the injection of blue ink and then decapitated. Only animals with confirmed microinjection into right lateral ventricle were included in the study.

Experimental procedure

All drugs were injected into the right lateral ventricle of freely moving rats in a volume of 1 µl during 60 s. A stainless-steel needle was placed into the guide cannula and connected by a silicone catheter to a Hamilton microliter syringe. The injection cannula was left placed for an additional minute to avoid reflux. On the proestrus morning different groups of rats (experimental groups, n = 8 animals/group) received a single i.c.v. injection of (1) allopregnanolone (6 µM), (2) bicuculline (9.8 µM), (3) bicuculline plus allopregnanolone, or (4) vehicle. Twenty four hours after, estrus morning (09:00 h), the animals were decapitated. The ovaries were removed and cleaned free of fat and their ovi-

ducts were flushed with saline solution. The number of oocytes present was counted with the aid of a dissecting microscope [24]. After that, 1 ovary was processed for routine optic microscopy (hematoxilin-eosin staining), whereas the other ovary was enucleated and processed for electron microscopy.

Morphological analysis: light and electron microscopy

For light microscopy, the ovaries were placed overnight at room temperature in a solution 10% phosphate buffered neutral formalin, dehydrated in ethanol series, cleared in xylene, and embedded in paraffin. Serial paraffin sections 5 µm thick were mounted on 3-aminopropytriethoxysilane (Sigma Chemical Co., St. Louis, MO, USA) coated slides and used for routine hematoxylin and eosine staining. The tissue samples were observed and photographed with Zeiss IM 35 microscope. For electron microscopy, the ovaries were fixed for 1 h in 5% glutaraldehyde (TAAB) in 0.1 M cacodylate buffer (pH 7.2–7.4). They were post-fixed in 1% osmium tetroxide and 1% potassium ferrocyanide in the same buffer for 4 h, dehydrated in a series of cold (4 °C) graded acetone, and embedded in Araldite. Thin sections, were cut with a diamond knife in a Porter-Blum MT-2 ultra microtome, and examined using a Siemens Elmiskop 1A electron microscope.

Hormone assays

Animals were killed by decapitation. Trunk blood was collected for each experimental group in heparinized tubes and centrifuged during 15 min at 3 000 rpm (Beckman TJ-6RS). The plasma obtained was kept frozen (−30 °C) until hormone assays were run. Serum LH and PRL were determined by RIA using kits supplied by the National Hormone Pituitary Program, USA. The LH standard was NIDDK-rLH-RP-3 and the antibody NIDDK antibody rLH-S-11, and PRL standard was NIDDK-rPRL-RP-3 and the antibody NIDDKantibody-rPRL-S-9. The sensitivity of the assay was 0.5 ng/tube. The intra- and inter-assay coefficients of variation were 9% and 11%, respectively. The data were expressed in nanograms per milliliter of serum in terms of NIDDK-rLH-RP-1 and NIDK-rPRL-RP-3 reference preparation. Progesterone concentrations were measured using a commercially obtained kit (Diagnostic Products Corporation, LA, CA, USA). The sensitivity of the assay was 0.02 ng/ml, and the inter- and intra-assay coefficients of variation were 5% and 6%, respectively.

Counting of apoptotic cells

Apoptotic cells were counted in hematoxilin-eosin stained tissue sections following the procedure described by Van Dschepop HA et al. [25] and Telleria CM et al. [26]. A microscope with a 100× objective was used for analysis in each corpus luteum for the presence of fragmented nuclei. Only cells with advanced signs of apoptosis (containing multiple nuclear fragments) were selected. Four corpora lutea per animal were analyze, and approximately 500 cells per corpus luteum were studied. The results were expressed as a number of apoptotic nuclei per high-power field.

In situ detection of apoptosis

Nuclei exhibiting DNA fragmentation were detected by using the Death End Colorimetric Apoptosis System (Promega, Madison, WL, USA), which end-levels the fragmented DNA of apoptotic cells using a modified TUNEL (TdT-mediated the UTP Nick-End Labeling) test. This kit was used according to the instructions of the supplier with slight modifications. Paraffin-embedded sections were the deparaffinized 5 min in xylene, washed in 100% v/v ethanol for 5 min at room temperature, and rehydrated by sequentially immersing the slices through graded ethanol washes (95% v/v and 70% v/v) for 5 min each at room temperature. Slides were then washed in distilled water for 5 min at room temperature and fixed in 10% v/v phosphate buffer neutral formalin for 15 min at room temperature. Sections were then washed twice in TBS for 52 min at room temperature and subjected to proteinase K treatment (20 µg/ml) in TBS for 15 min. Tissue sections were washed in TBS for 5 min and refixed in 10% v/v phosphate buffered neutral formalin for 5 min. Sections were washed, incubated with equilibration buffer for 10 min, and treated with the TUNEL reaction mixture containing terminal deoxynucleotidyl transferase (TdT) and biotinylated nucleotides for 60 min at 37 °C. When the reaction was terminated, the slides were washed twice in fresh TBS for 5 min at room temperature, and the endogenous peroxidases blocked in 3% hydrogen peroxide for 5 min at room temperature. After a new TBS wash, sections were incubated with streptavidin conjugated with horse-radish peroxides (HRP) for 30 min at room temperature. Negative controls were incubated with label solution lacking TdT. Afterwards, 3′ end-bound streptavidin-HRP was visualized by incubation with diaminobenzidine (DAB) according to manufacturer's instructions. Finally, sections were counterstained with hematoxylin, mounted, and analyzed with light microscopy.

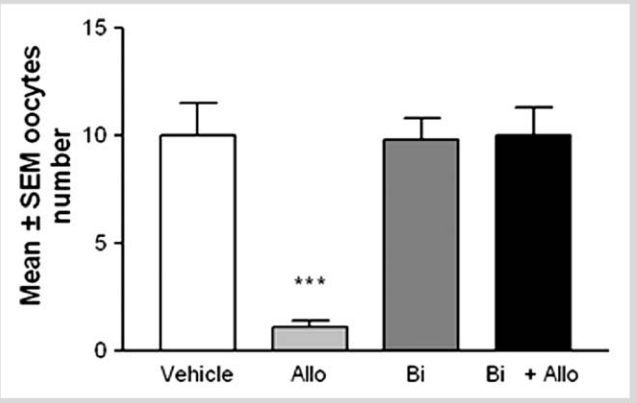

Fig. 1 Effects of allopregnanolone on the number of oocytes obtained from cycling rats injected with vehicle, Allo, Bi, and Bi + Allo (Allo: allopregnanolone, Bi: bicuculline). Results are expressed as mean ± SEM of 8 animals per experimental group from respective control. ***p<0.001.

Statistical analysis

Comparisons between the means of 2 groups were carried out using the Student *t*-test. For multiple comparisons, One-Way Analysis of Variance (ANOVA I) followed by a post-hoc Newman-Keuls test was used data were expressed as means ± SEM; $p < 0.05$ was considered as minimum criterion for assigning statistical significance.

Results

Effect of allopregnanolone on the oocytes number

Allopregnanolone injection induced a significant decrease in the number of oocytes in oestrous rats in comparison with the other groups ($p < 0.001$, **Fig. 1**).

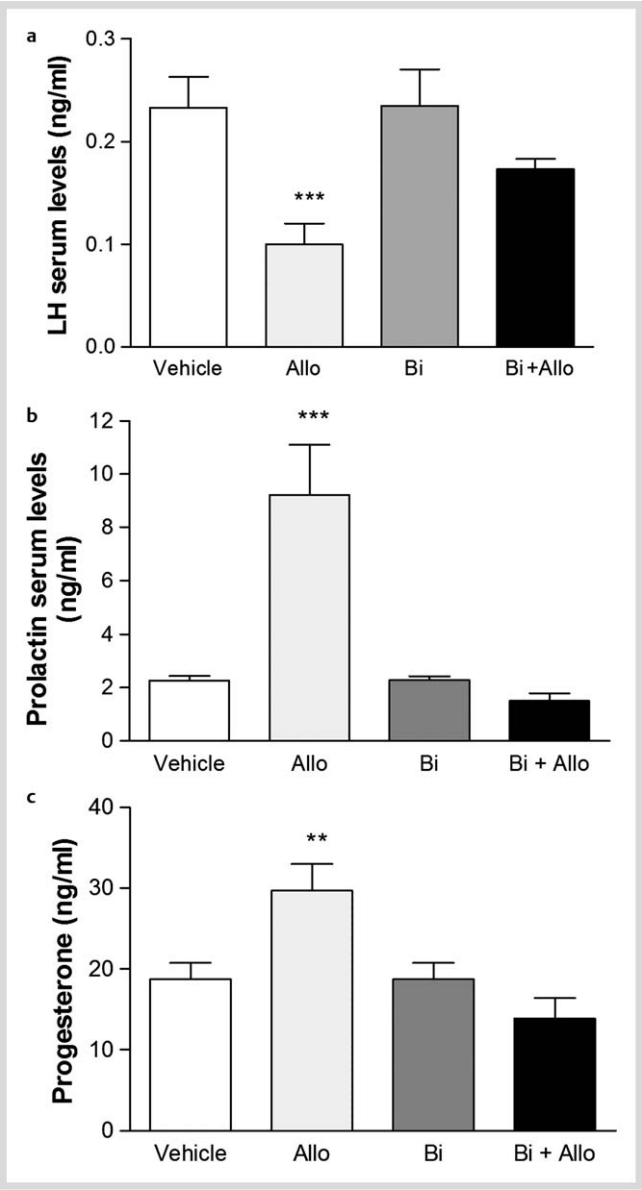

Fig. 2 Effect of allopregnanolone on luteinizing hormone **a**, prolactin **b**, and progesterone **c** serum levels in estrous rats after i.c.v. injection with vehicle, Allo, Bi, and Bi + Allo into right lateral ventricle. Results are expressed as means ± S.E.M. of 8 rats per experimental group. ***p<0.001 **p<0.01.

Effect of allopregnanolone on luteinizing hormone, prolactin, and progesterone serum levels

Allopregnanolone injection induced a significant decrease in LH serum concentrations compared with those observed in the control group (p<0.01, **Fig. 2a**). The injection of bicuculline prior to allopregnanolone reverted the effect induce by allopregnanolone (p<0.01). The injection of bicuculline alone did not altered LH serum levels compared with those observed in the control group (**Fig. 2a**). The prolactin serum level was significantly increased in animals injected with allopregnanolone compared with those observed in the control group (p<0.001). The injection of bicuculline prior to allopregnanolone reverted this effect (p<0.001). The injection of bicuculline alone did not altered prolactin serum levels as it was observed in the control group (**Fig. 2b**). Serum progesterone levels were significantly increased by allopregnanolone injection compared with those observed in the control group, (p<0.05). The injection of bicuculline prior to allopregnanolone reverted the effect induced by allopregnanolone (p<0.001). The injection of bicuculline alone did not altered progesterone serum levels compared with those observed in the control group (**Fig. 2c**).

Effect of allopregnanolone on the ovarian histology (light microscopy)

Under the light microscopy, a lot of apoptotic cells were evident in corpora lutea of rats injected with vehicle (**Fig. 3A** upper panel), bicuculline (**Fig. 3C**) or bicuculline plus allopregnanolone (**Fig. 3D**). The apoptotic cells showed a single small densely stained nucleus with pyknotic appearance or nuclei containing marginated chromatin or cells containing multiple, smaller, densely staining nuclear fragments. In addition, the apoptotic cells displayed an intense cytoplasmic eosinophilic stain. Numerous apoptotic cells were detected in the control group injected with vehicle (**Fig. 3A**). Animals injected with allopregnanolone (**Fig. 3B**) exhibit steroidogenic cells in corpora lutea that consisted primarily of large nucleated polyhedral cells with abundant cytoplasm and regular circular nuclei with dispersed chromatin and 1 or 2 nucleoli. The numbers of apoptotic cells were significantly decreased after the i.c.v. allopregnanolone administration (**Fig. 3B**). Whereas allopregnanolone injected animals had an average of 2.7±0.9 (n=190) apoptotic cells per each high power field, rats receiving the vehicle showed an average of 23.5±2.5 (n=200) (p<0.001) (**Fig. 3A**). There were no differences in the number of apoptotic cells with the bicuculline (**Fig. 3C**) and bicuculline plus allopregnanolone (**Fig. 3D**) in treated groups compared with the control group.

Effect of allopregnanolone on the corpora lutea apoptosis

The apoptotic nature of the cells displaying fragmented nuclei by routine staining was validated by using and in situ 3′ end labeling (apoptosis detection system-TUNEL). The results were expressed as the mean±SEM of the number of apoptotic cells per 10 high-power fields in corpora lutea obtained from estrous rats. The number of apoptotic nuclei were significantly lower in allopregnanolone treated group in comparison with vehicle (p<0.001) (**Fig. 3**, lower panel).

Effect of allopregnanolone on the ovarian histology (electron microscopy)

The morphological changes observed by light microscopy were confirmed by electron microscopy to study the ultra structural features of the apoptotic cells. Ultrastructurally, the cells from vehicle injected rats (**Fig. 4A, B, C**), which were undergoing apoptosis exhibit a heavily deteriorated smooth endoplasmic reticulum with distention and fragmentation. The luteal cells undergoing apoptosis exhibits their cytoplasm organization heavily deteriorated due to organelle swelling and fragmentation, mainly of the smooth endoplasmic reticulum. The nuclei in luteal regression displayed marked chromatin condensation and a prominent perinuclear space. In a more advanced degree, large and irregular aggregates of condensed chromatin are seen in apoptosis. After fragmentation of the nucleus, chromatin aggregates were observed among cytoplasmic residues, condensing into apoptotic bodies. The well preserved corpus luteum cells observed from allopregnanolone injected rats (**Fig. 4D, E, F**) contain a large round nucleus, with dispersed chromatin, and 1 or 2 nucleoli, a Golgi complex, a moderate amount of rough

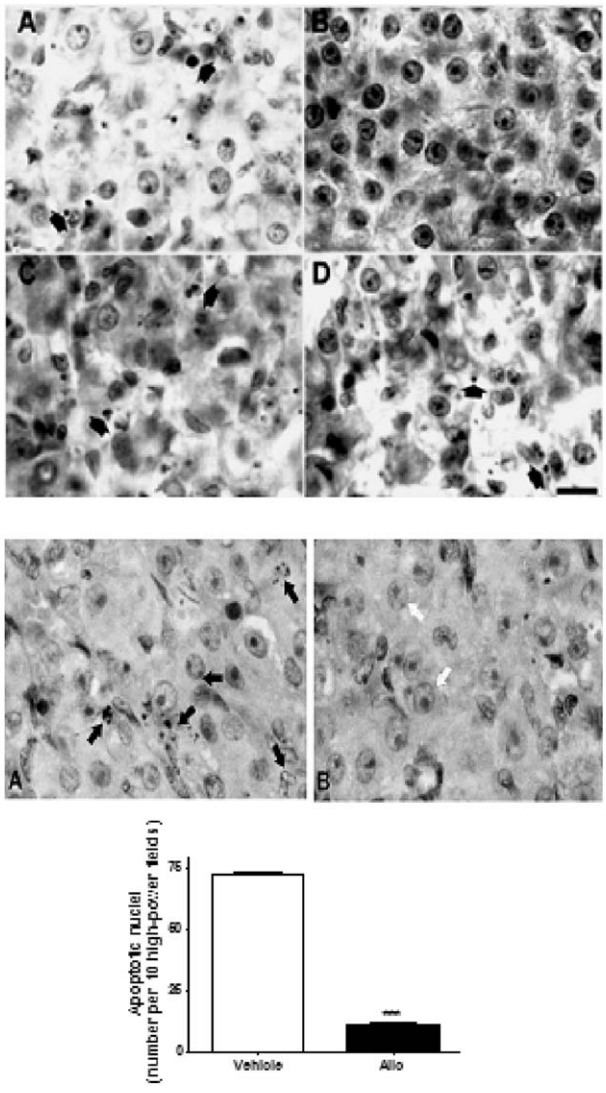

Fig. 3 Upper panel: Light micrographs of corpora lutea cells from rats after treatment with vehicle **A**, Allo **B**, Bi **C**, and Bi + Allo **D**. Short black arrows point to apoptotic bodies in panels **A**, **C**, and **D**. Allo: allopregnanolone, Bi: bicuculline. Bar inset in panel **D** = 35 µm. Lower panel: Light micrographs of corpora lutea cells using in situ 3′ end labeling (TUNEL) after treatment with vehicle **A**. Black arrows indicate small densely stained nucleus, nuclei with marginated chromatin and cells with nuclear fragments and after treatment with Allo **B**. White arrows indicate healthy luteal cells.

endoplasmic reticulum, abundant smooth endoplasmic reticulum, and the numerous pleomorphic mitochondria characteristic of steroidogenic cells.

Discussion

In the present work, we have shown that central administration of allopregnanolone in proestrous intact rats significantly interfered with the luteal regression process. This was demonstrated by the finding that after 24 h of centrally administered allopregnanolone there was a decrease in the number of apoptotic cells. A significant amount of apoptotic cells has been identified in the regressing corpora lutea of the vehicle injected rats in contrast with corpora lutea of animals injected with allopregnanolone,

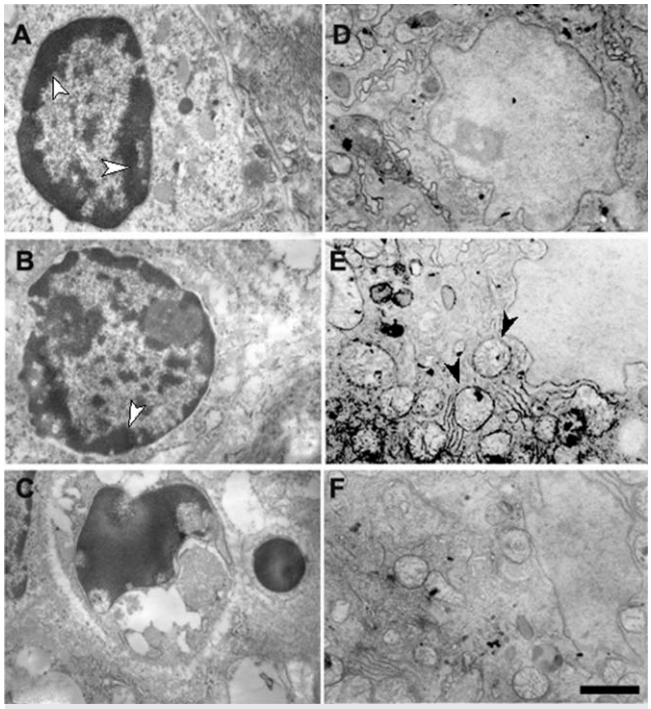

Fig. 4 Electron micrographs of the corpora lutea cells from vehicle treated rats (Micrographies **A**, **B** and **C**, white head arrows) show a progressive chromatin clumping during the physiological apoptotic process. See also the cytoplasm organelle reduction and alterations especially in the advanced stage. Micrographies **D**, **E**, and **F** show luteal cells from allopregnanolone treated animals (black head arrows). Observe the even chromatin distribution and the perfectly well preserved organelles. Bar inset in panel **C** = 400 µm.

where an inhibition of apoptotic process was found. Also, the ovulation and the LH release were inhibited and the progesterone and prolactin serum levels were augmented. These results show that the central effect allopreganolone over reproductive function could be due to a centrally originated LH mediated effect over ovarian function, that affect luteal regression, through the inhibition of apoptosis and stimulation of progesterone and prolactin release.

Despite the known effect of LH on the corpus luteum as luteotropic factor, it also has been proposed that LH may have a deleterious effect on the decreasing luteal function during the cycle and at the end of pregnancy [27, 28]. LH also inhibits the conversion of pregnenolone to progesterone and stimulates the 20α-HSD activity and gene expression [28]. According to our results, an inhibition of LH serum levels could be responsible of the increase in serum progesterone levels.

Recently, Micevych et al. [29] have shown that progesterone produced in the central nervous system (neuroprogesterone) is capable of positively regulating the release of LH. This concept reinforces our hypothesis about the actions of neurosteroids as potent modulators of neuroendocrine regulatory systems capable of producing changes at the peripheral glands like ovary.

Studies to date have demonstrated that luteolysis is a process highly regulated by hormones [18, 30–33] and because apoptosis is associated with luteolysis, it is assumed that apoptotic cell death in the corpus luteum is a hormonally regulated process. Luteal regression in the rat is a complex process that involves 2 stages: the first named functional regression is characterized by a rapid decrease in the capacity of the gland to produce and

secrete progesterone. The second, called structural regression, is characterized by a decrease in the weight and size of CL, which regresses within the ovarian stroma and is known as corpus albicans, which finally is reabsorbed and replaced by ovarian stroma. The main mechanism involved in structural regression is the removal of luteal cells by apoptosis [34]. The process occurs not only because of the luteal cell death but also due to the replacement of the vascular supply and supporting connective tissue, bundles of collagen fibers, fibroblasts, and macrophages [19]. It is highly possible that luteal progesterone protects the corpora lutea from the apoptotic process as it does in other tissues such as the uterine epithelium and the mammary gland [35]. Also, progesterone can protect corpus luteum from luteolysis according to stage of the oestrous cycle [18,26,34]. The results presented here could indicate that the anti-apoptotic effect of allopregnanolone (the main progesterone metabolite) in rats may be a consequence of the central inhibition of LH serum levels and with an increase in progesterone and prolactin serum levels [4,30].

Then, centrally administered allopregnanolone could protect corpora lutea of the luteal regression, through inhibiting apoptosis even while structural regressing began. Moreover, it could interfere with functional luteolysis through prolonging the survival of the corpus luteum with an increase of progesterone secretion.

The mechanisms that regulate receptivity, LH surge and ovulation involve an interaction between ovarian steroids and different neurotransmitter pathways, such as GABA [36–38]. Both effects could be mediated through $GABA_A$ receptors, because they were completely blocked by bicuculline. In a previous report, we have shown that allopregnanolone induces changes in dopamine and GABA activity in preoptic area and medial basal hypothalamus [4,15]. We suggest that these changes may be involved in the inhibition of sexual receptivity and/or gonadotrophin release.

Also, it is known that allopregnanolone suppresses hypothalamic GnRH release in vitro and this effect is mediated by an interaction with the GABA receptors [4,13,17,38]. In this work allopregnanolone inhibited LH surge through $GABA_A$ receptors and this effect has as a consequence changes in luteinization. These effects may be exerted at the level of afferent nerve terminals that regulate the GnRH secretion [15,37,39–42].

On the other hand, prolactin, one of the main hormones involved in the corpus luteum function in rats, acts as luteotrophic hormone maintaining the structural and functional integrity of the corpus luteum for 6 days after mating. Prolactin, can rescue the corpus luteum in the morning of diestrum 2 day when the capacity of the gland to produce progesterone is maximal [43]. The luteotrophic action of prolactin is characterized by an enhanced progesterone secretion, which is essential for the implantation of fertilized ovum, maintenance of pregnancy, and inhibition of ovulation [44].

Moreover, it is well known that dopamine release from nervous in the medial basal hypothalamus is a potent inhibitory signal regulating prolactin secretion [45]. Previous studies from our laboratory have demonstrated that allopregnanolone inhibited the dopamine turnover from hypothalamus in female rat [15]. In this work, our data have shown an increase in prolactin serum levels suggesting a central effect of allopregnanolone on the hypothalamic dopaminergic system that could be the responsible of the increase in prolactin levels and corpus luteum rescue.

In conclusion, our findings show that allopregnanolone was fully effective suppressing LH secretion and, in consequence inhibiting luteinization. This effect could be mediated by interaction with hypothalamic neuronal network, specifically the GABAergic system. An involvement of GABA and dopamine system in the regulation of allopregnanolone effect is also supported by studies showing that the same dose caused a decrease in hypothalamic DA/DOPAC turnover that stimulate a prolactin secretion [15]. In consequence, at the ovary level, the suppressive effects observed on the corpora lutea apoptotic process may also be mediated by its ability to inhibit LH at pituitary level, promoting progesterone release from CL in response to prolactine luteotropic action.

In summary, the results of this study have established that central administration of allopregnanolone affects luteal regression through inhibition of apoptosis, regulating the neuroendocrine system involved in this process. The physiological significance of these findings shows that allopreganolone was able to promote corpus luteum survival by multiple prosurvival neuroendocrine signaling pathways as luteinizing hormone, progesterone, and prolactin level modifications that are modulated by specific hypothalamic and pituitary neural networks.

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References

1 *McEwen BS*. Non-genomic and genomic effects of steroids on neural activity. Trends Pharmacol Sci 1991; 12: 141–147
2 *Melcangi RC*, *Panzica G*, *Garcia-Segura LM*. Neuroactive steroids: focus on human brain. Neuroscience 2011; 191: 1–5
3 *Micevych P*, *Sinchak K*. Synthesis and function of hypothalamic neuroprogesterone in reproduction. Endocrinology 2008; 149: 2739–2742
4 *Laconi MR*, *Casteller G*, *Gargiulo P*, *Bregonzio C*, *Cabrera RJ*. The anxiolytic effect of Allopregnanolone is associated with gonadal hormonal status in female rats. Eur J Pharmacol 2001; 417: 111–116
5 *Robel P*, *Baulieu EE*. Neurosteroids: Biosynthesis and function. Trends Endocrinol Metab 1994; 5: 1–8
6 *Majewska MD*. Neurosteroids: endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. Prog Neurobiol 1992; 38: 379–395
7 *Paul SM*, *Purdy RH*. Neuroactive steroids. FASEB J 1992; 6: 2311–2322
8 *Guidotti A*, *Costa E*. Can the antidysphoric and anxiolytic profiles of selective serotonin reuptake inhibitors be related to their ability to increase brain 3 alpha, 5 alpha-tetrahydroprogesterone (Allopregnanolone) availability? Biol Psychiatry 1998; 44: 865–873
9 *Smith SS*, *Gong QH*, *Moran MH*, *Bitran D*, *Frye CA*, *Hsu FC*. Withdrawal from 3alpha-OH-5alpha-Pregnan-20-One using a pseudopregnancy model alters the kinetics of hippocampal GABAA-gated current and increases the GABAA receptor alpha4 subunit in association with increased anxiety. J Neurosci 1998; 18: 5275–5284
10 *Smith SS*, *Gong QH*, *Hsu FC*, *Markowitz RS*, *French-Muller JM*, *Li X*. GABA(A) receptor alpha4 subunit suppression prevents withdrawal properties of an endogenous steroid. Nature 1998; 392: 926–930
11 *Monnet FP*, *Maurice T*. The sigma1 protein as a target for the non-genomic effects of neuroactive steroids: molecular, physiological, and behavioral aspects. J Pharmacol Sci 2006; 100: 93–118
12 *Schumacher M*, *Coirini H*, *Robert F*, *Guennoun R*, *El-Eltr M*. Genomic and membrane actions of progesterone: implications for reproductive physiology and behavior. Behav Brain Res 1999; 105: 37–52
13 *Lambert JJ*, *Belelli D*, *Hill-Venning C*, *Peters J*. Neurosteroids and $GABA_A$ receptor function. Trends Pharmacol Sci 1995; 16: 295–303
14 *Micevych P*, *Sinchak K*, *Mills RH*, *Tao L*, *LaPolt P*, *Lu JK*. The luteinizing hormone surge is preceded by an estrogen-induced increase of hypothalamic progesterone in ovariectomized and adrenalectomized rats. Neuroendocrinology 2003; 78: 29–35

15 *Laconi MR*, *Cabrera RJ*. Effect of centrally injected Allopregnanolone on sexual receptivity, luteinizing hormone release, hypothalamic dopamine turnover, and release in female rats. Endocrine 2002; 17: 77–83
16 *Cruz ME*, *Villegas G*, *Domínguez-González A*, *Chavira R*, *Domínguez R*. Ovulation delay induced by blockade of the cholinergic system on dioestrus-1, is related to changes in dopaminergic activity of the preoptic anterior-hypothalamic area of the rat. Brain Res Bull 2001; 54: 339–344
17 *Giuliani FA*, *Yunes R*, *Mohn CE*, *Laconi M*, *Rettori V*, *Cabrera R*. Allopregnanolone induces LHRH and glutamate release through NMDA receptor modulation. Endocrine 2011; 40: 21–26
18 *Stocco C*, *Telleria C*, *Gibori G*. The molecular control of corpus luteum formation, function, and regression. Endocr Rev 2007; 28: 117–149
19 *Goyeneche AA*, *Deis RP*, *Gibori G*, *Telleria CM*. Progesterone promotes survival of the rat corpus luteum in the absence of cognate receptors. Biol Reprod 2003; 68: 151–158
20 *McGuire WJ*, *Juengel JL*, *Niswender GD*. Protein kinase C second messenger system mediates the antisteroidogenic effects of prostaglandin F2 alpha in the ovine corpus luteum in vivo. Biol Reprod 1994; 51: 800–806
21 *Pate JL*. Cellular components involved in luteolysis. J Anim Sci 1994; 72: 1884–1890
22 *Barbaccia ML*, *Roscetti G*, *Bolacchi F*, *Concas A*, *Mostallino MC*, *Purdy RH*, *Biggio G*. Stress-induced increase in brain neuroactive steroids: antagonism by abecarnil. Pharmacol Biochem Behav 1996; 54: 205–210
23 *Paxinos G*, *Watson C*. The rat brain in stereotaxic coordinates. London: Academic Press, 2009
24 *Morales L*, *Chávez R*, *Ayala ME*, *Domínguez R*. Effects of unilateral or bilateral superior ovarian nerve section in prepubertal rats on the ovulatory response to gonadotrophin administration. J Endocrinol 1998; 158: 213–219
25 *Van de Schepop HA*, *de Jong JS*, *van Diest PJM*, *Baak JP*. Counting of apoptotic cells: a methodological study in invasive breast cancer. Clin Mol Pathol 1996; 49: 214–217
26 *Telleria CM*, *Goyeneche A*, *Cavicchia JC*, *Statu AO*, *Deis RP*. Apoptosis induced by antigestagen RU486 in rat corpus luteum of pregnancy. Endocrine 2001; 15: 147–155
27 *Plas-Roser S*, *Muller B*, *Aron C*. Estradiol involvement in the luteolytic action of LH during estrous cycle in the rat. Exp Clin Endocrinol 1988; 92: 145–153
28 *Stocco CO*, *Chedrese J*, *Deis RP*. Luteal expression of cytochome P450 side-chain cleavage, steroidogenic acute regulatory protein, 3β-hydroxysteroid dehydrogenase, and 20α-hydroxysteroid dehydrogenase genes in late pregnant rats: effect of luteinizing hormone and RU486. Biol Reprod 2001; 65: 1114–1119
29 *Micevych P*, *Bondar G*, *Kuo J*. Estrogen actions on neuroendocrine glia. Neuroendocrinology 2010; 91: 211–222
30 *Wang Z*, *Tamura K*, *Yoshie M*, *Tamura H*, *Imakawa K*, *Kogo H*. Prostaglandin F2alpha-induced functional regression of the corpus luteum and apoptosis in rodents. J Pharmacol Sci 2003; 92: 19–27
31 *Peluffo MC*, *Young KA*, *Stouffer RL*. Dynamic expression of caspase -2, -3, -8, and -9 proteins and enzyme activity, but not messenger ribonucleic acid, in the monkey corpus luteum during the menstrual cycle. J Clin Endocrinol Metab 2005; 90: 2327–2335
32 *Peluffo MC*, *Bussman L*, *Stouffer RL*, *Tesone M*. Expression of caspase -2, -3, -8 and -9 proteins and enzyme activity in the corpus luteum of the rat at different stages during the natural estrous cycle. Reproduction 2006; 132: 465–475
33 *Telleria CM*. Can luteal regression be reversed? Reprod Biol Endocrinol 2006; 4: 53
34 *Goyeneche AA*, *Calvo V*, *Gibori G*, *Telleria CM*. Androstenedione interferes in luteal regression by inhibiting apoptosis and stimulating progesterone production. Biol Reprod 2002; 66: 1540–1547
35 *Feng Z*, *Marti A*, *Jehn B*, *Altermatt HJ*, *Chicaiza G*, *Jaggi R*. Glucocorticoid and progesterone inhibit involution and programmed cell death in the mouse mammary gland. J Cell Biol 1995; 131: 1095–1103
36 *Tellería CM*, *Carrizo DG*, *Deis RP*. Levonorgestrel inhibits luteinizing hormone-stimulated progesterone production in rat luteal cells. J Steroid Biochem Mol Biol 1994; 50: 161–166
37 *Chu Z*, *Moenter SM*. Endogenous activation of metabotropic glutamate receptors modulates GABAergic transmission to gonadotropin-releasing hormone neurons and alters their firing rate: a possible local feedback circuit. J Neurosci 2005; 25: 5740–5749
38 *Calogero AE*, *Palumbo MA*, *Bosboom N*, *Burrello N*, *Ferrara E*, *Palumbo G*, *Petraglia F*, *D'Agata R*. The neuroactive steroid Allopregnanolone suppresses hypothalamic gonadotropin-releasing hormone release through a mechanism mediated by the gamma-aminobutyric acid A receptor. J Endocrinol 1998; 158: 121–125
39 *Lin YS*, *Li XF*, *Shao B*, *Hu MH*, *Goundry AL*, *Jeyaram A*, *Lightman SL*, *O'Byrne KT*. The role of GABAergic signaling in stress-induced suppression of gonadotrophin-releasing hormone pulse generator frequency in female rats. J Neuroendocrinol 2012; 24: 477–488
40 *Genazzani AR*, *Palumbo MA*, *deMicheroux AA*, *Artini PG*, *Criscuolo M*, *Ficarra G*, *Guo AL*, *Benelli A*, *Bertolini A*, *Petraglia F*, *Purdy RH*. Evidence for a role for the neurosteroid Allopregnanolone in the modulation of reproductive function in female rats. Eur J Endocrinol 1995; 133: 375–380
41 *Smith M*, *Jennes L*. Neural signals that regulate GnRH neurons directly during the oestrous cycle. Reproduction 2001; 122: 1–10
42 *Timby E*, *Hedström H*, *Bäckström T*, *Sundström-Poromaa I*, *Nyberg S*, *Bixo M*. Allopregnanolone, a GABAA receptor agonist, decreases gonadotropin levels in women. A preliminary study. Gynecol Endocrinol 2011; 27: 1087–1093
43 *Freeman ME*, *Kanyicska B*, *Lerant A*, *Nagy G*. Prolactin: Structure, Function, and Regulation of Secretion. Physiological Reviews 2000; 80: 1523–1631
44 *Michael AE*, *Abayasekara DR*, *Webley GE*. Cellular mechanisms of luteolysis. Mol Cell Endocrinol 1994; 99: 1–9
45 *Kennett JE*, *McKee DT*. Oxytocin: An emerging regulator of prolactin secretion in the female rat. J Neuroendocrinol 2011; 10: 1365–2826