Involvement of the coeliac ganglion in the luteotrophic effect of androstenedione in late pregnant rats

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Abstract

Using the ex vivo coeliac ganglion-superior ovarian nerve-ovary system at the end of pregnancy when luteal regression starts, we investigated whether, when administered systemically or when added directly to the ganglion compartment, androstenedione (A2) can reverse such regression, and whether the neural (noradrenaline (NA)) and endocrine (A2) joint action modifies the release of ovarian progesterone. The experimental groups were as follows: group 1 – A2 injected systemically 48 h before incubation of the system (A2s); group 2 – A2 directly added to the ganglion compartment (A2g); group 3 – A2 injected 48 h before incubation of the system with NA in the ganglion compartment (A2 + NA); group 4 – A2 plus NA added to the ganglion compartment (NA + A2g). The controls were ex vivo systems without treatment (control), and with the addition of NA alone in the ganglion compartment (NA). The results were as follows. For (A2s) versus control, progesterone increased on days 19 and 21 of pregnancy at all the studied times and only at 180 min on day 20. For (A2 + NA) versus (A2s), progesterone increased on days 19 and 21. For (A2 + NA) versus NA, progesterone increased at all the studied times on days 19 and 21 and at 180 min on day 20. For (A2g) versus control, progesterone significantly increased every pregnancy day. For (NA + A2g) versus (A2g), progesterone decreased at 120 and 180 min on day 19. For (NA + A2g) versus NA, progesterone increased on days 20 and 21. We can conclude that A2 can reverse the functional regression of the corpus luteum either systemically or, what is more surprising, when directly added to the coeliac ganglion, whose action on the ovary is exerted via superior ovarian nerve.

Reproduction (2006) 131 361–368

Introduction

The coeliac ganglion, part of the sympathetic prevertebral chain, is constituted mainly by principal neurons and inter-neurons such as the peptidergic and the intensely fluorescent (SIF) (Eränkö 1978, Abe et al. 1983, Matthews 1989, Prud’Homme et al. 1999). The sympathetic ganglia possess a great variety of specific receptors and neurotransmitters, among them catecholamines (Klein & Burden 1988), neuropeptides (Dalsgaard et al. 1983) and gaseous neurotransmitters such as nitric oxide (Morales et al. 1995). It is interesting to point out the presence of a profuse capillary plexus that constitutes a microcirculation among the different ganglionic structures (Chau & Lu 1995).

Previous studies have shown that the humoral environment influences the functioning of the coeliac ganglion, thus affecting the ovary via the superior ovarian nerve (Aguado 2002).

The superior ovarian nerve is mostly constituted by adrenergic fibres whose origin neurons are mainly located in the coeliac ganglion (Baljet & Drukker 1979) and whose terminals act directly upon the secretory part of the ovary, including the theca externa and interna of the follicle, the corpus luteum capsule and the interstitial gland. However, direct contact of nerves with luteal and granulosa cells has not been shown (Lawrence & Burden 1980, Erickson et al. 1985, Klein & Burden 1988). The ovarian noradrenergic stimulation affects progesterone secretion and its synthesis by an increase of cytochrome P450scC and 3β-HSD enzymatic activity. In corpus luteum, this effect is mediated via β1 and β2 receptors (Pesta et al. 1994, Miszkiel & Kotwica 2001) with activation of cAMP (Lefkowitz, 1987). Besides acting on the luteal β-receptors, noradrenaline (NA) acts on ovarian vascular adrenoreceptors (Reynolds & Ford 1984) and adipose cell β-receptors. The stimulation of lipolysis and the increased blood flow through the ovary could supply the corpus luteum with serum-derived lipoproteins as a source of cholesterol for luteal steroidogenesis (Williams 1989). In studies in conscious heifers, the infusion of NA into the abdominalis aorta stimulated the secretion of progesterone within a few minutes in a dose-dependent manner (Kotwica et al. 2002). De Bortoli et al. (1998, 

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ISSN 1470–1626 (paper) 1741–7899 (online)
DOI: 10.1530/rep.1.00852
Online version via www.reproduction-online.org
2000) demonstrated that a central adrenergic stimulus produces acute effects on the release of steroids from the ovary. Ovarian progesterone changes could be attributed to signals coming from ganglionic neurons, which are affected by the central adrenergic stimulation. By use of the ex vivo coeliac ganglion-superior ovarian nerve-ovary system (coeliac ganglion-SON-ovary) of rats in the second half of pregnancy, the release of ovarian progesterone was modified at 30 min of incubation with NA in the ganglion compartment (Casais et al. 2001). This rapid ovary response was probably the result not only of a direct influence of NA on the luteal cells via β-receptors, but also of other factors released via SON which could amplify this effect indirectly.

It is already known that progesterone is a survival factor in the corpora lutea (Sugino et al. 1997, Tellería et al. 1999, Villavicencio et al. 2002). At the end of pregnancy, the corpora lutea suffer a regression process that decreases their capacity to produce progesterone (functional regression) and causes the subsequent involution of the luteal tissue, including steroidogenic and nonsteroidogenic cells (structural regression) (Bowen-Shauver & Tellería 2003).

On the other hand, androstenedione (A2), the principal androgen in rat, has a well-known antiapoptotic affect either through its intraluteal conversion to oestradiol (Gibori et al. 1988) or through a non-genomic mechanism (Carrizo et al. 1994, Thordarson et al. 1997, Machelón et al. 1998). It has also been suggested that A2 could have a dual effect: a non-genomic effect that would lead to the activation of a complex cascade of signal transduction (Cato & Peterziel 1998) and a subsequent genomic event.

With these antecedents, we thought it interesting to investigate whether A2 is capable of reversing the luteal functional regression, whether systemically administered (by injection 48 h before the system incubation) or directly added to the ganglion compartment. Considering that NA action in the ganglion compartment decreases as pregnancy progresses (Casais et al. 2001) and that A2 inhibits the luteal functional regression, we also decided to analyse the neural and endocrine joint action of NA and A2 on the release of ovarian progesterone at the end of rat pregnancy.

**Materials and Methods**

**Animals**

Adult female rats bred in our laboratory (originally Holtzman albino strain) aged 2–3 months and weighing 220 ± 50 g were used. The rats were housed under controlled light (lights on from 0700 to 1900 h) and temperature (24 ± 2 °C) conditions, and allowed ad libitum access to water and food (rat chow-Cargil SACI; Saladillo, Buenos Aires, Argentina).

To induce pregnancy, female rats were caged individually with fertile males beginning on the afternoon of prooestrus. Positive mating was verified on the following morning by identifying sperm or copulation plugs in the vagina. This day was designated as day 0 of pregnancy. In our laboratory, rats usually give birth on day 22.

Animals were handled according to the procedures approved in the UFAW Handbook on the Care and Management of Laboratory Animals, vol. 1: Terrestrial Vertebrates (7th edn), edited by T Poole (1999), and the Guide for Animal Use and Handling of the National University of San Luis.

**Systemic A2**

A2 was systemically administered 48 h before the incubation of coeliac ganglion-SON-ovary system with and without addition of NA in the ganglion compartment.

**Drugs**

Drugs used were:

- hydrochloride of L-D-NA (Sigma)
- 4-androsteno-3,17,diona (A2) (Sigma).

**A2 preparation for injection**

A2 was dissolved in benzilic alcohol (2–5% of the final volume) to a sufficient concentration to inject each animal with 10 mg A2/0.2 ml vegetable oil. The administration to animals was subcutaneous (s.c.).

**Procedure**

Groups of six animals each on days 17–19 of pregnancy were used for each experimental procedure with the following distribution:

- group 1: pregnant rats on day 17
- group 2: pregnant rats on day 18
- group 3: pregnant rats on day 19

Rats were administered (s.c.) 10 mg A2/0.2 ml oil vehicle. This procedure was carried out at 0900 h on the pregnancy days mentioned (days 17–19). The animals were caged individually, and, at 0900 h on days 19–21 of pregnancy, that is to say, 48 h after injecting A2, they were anaesthetized under ether bell, and the coeliac ganglion-SON-ovary system was immediately extracted (Casais et al. 2001).

The fetuses were removed and killed under ether anaesthesia. Briefly, the coeliac ganglion-SON-ovary system was placed in a cuvette with two compartments, each containing 2 ml Krebs-Ringer bicarbonate buffer, pH 7.4; 0.1 mg glucose/ml; and 0.1 mg albumin/ml. After 30-min preincubation, the liquid was removed from the cuvette, buffer was added to the ovarian compartment, and buffer plus 0.1 mg ascorbic acid/ml was added to the ganglion compartment. The previous description corresponds to the group (A2)s. Previous injection of A2 plus 10−7 mol NA/l in the ganglion compartment constituted the group (A2 + NA).
Periodic extractions of incubation liquid were made from the ovary (250 µL) at 30, 60, 120 and 180 min, and they were stored in a freezer at −20°C until the determination of progesterone by RIA. The corresponding corrections were made in all cases, taking into consideration the volume extracted in each tested period.

**A₂ in coeliac ganglion**

The coeliac ganglion-SON-ovary system was incubated in the presence of A₂ with or without the addition of NA to the ganglion compartment.

10⁻⁶ mol A₂/l preparation

A₂ was dissolved in benzilic alcohol (2–5% of the final volume), and a solution at final concentration of 10⁻⁶ mol A₂/l in Krebs-Ringer bicarbonate buffer, pH 7.4; 0.1 mg glucose/ml; and 0.1 mg albumin/ml was added directly to the ganglion compartment. This solution was used for group (A₂)g.

The solution (NA + A₂)g was prepared in such a way that the joint preparation of NA and A₂ constituted a solution with final concentration of 10⁻⁶ mol NA + A₂/l in Krebs-Ringer bicarbonate buffer, pH 7.4; 0.1 mg glucose/ml; and 0.1 mg albumin/ml. This solution was directly added to the ganglion compartment.

**Procedure**

Rats on days 19–21 of pregnancy were anaesthetized under ether bell, and the coeliac ganglion-SON-ovary system was extracted by the procedure previously described. The addition of 10⁻⁶ mol A₂/l plus 0.1 mg ascorbic acid/ml to the ganglion compartment characterized group (A₂)g. Group (NA + A₂)g had 10⁻⁶ mol NA + A₂/l plus 0.1 mg ascorbic acid/ml in the ganglion compartment. Progesterone determination in the ovarian incubation liquid was measured by RIA at all the studied times.

**Progesterone assay**

Progesterone was measured by RIA using antisera raised against progesterone-11-BSA conjugate in rabbits, provided by Dr R P Deis (Laboratorio de Reproducción y Lactancia Mendoza, Argentina). The sensitivity, variability and cross-reaction of this RIA have been previously reported (Bussmann & Deis 1979). The sensitivity of the assay is less than 5 ng progesterone/ml serum, and the inter- and intra-assay coefficients of variation were less than 10%. This assay has been previously validated (Bussmann & Deis 1979).

**Statistical analysis**

Results are presented as mean ± S.E.M. in each group of six rats. Differences between two groups were analysed with Student’s t-test. The analysis of the variance (ANOVA I), followed by Duncan’s multiple-range test was used for several comparisons. A value of P < 0.05 was considered statistically significant (Snedecor & Cochran 1976).

**Results**

**Systemic effect of A₂ on the release of ovarian progesterone in the coeliac ganglion-SON-ovary system**

Figure 1A–C shows that in the coeliac ganglion-SON-ovary system extracted from animals on days 19 and 21 of pregnancy, with previous s.c. administration (48 h) of 10 mg A₂/0.2 ml oil vehicle (A₂)s, the levels of progesterone increased significantly when compared with the control values at all the studied times (*P < 0.05). On day 20, a decrease at 30 and 60 min (0.06 ± 0.008 compared with 0.13 ± 0.02 ng/mg ovary, *P < 0.05; 0.08 ± 0.018 compared with 0.17 ± 0.03 ng/mg ovary, *P < 0.05 respectively) and an increase at 180 min (0.26 ± 0.02 compared with 0.18 ± 0.03 ng/mg ovary, *P < 0.05) were observed in relation to control values. The comparison was done with the control rats without treatment since the s.c. administration of 0.2 ml oil vehicle alone did not modify the release of progesterone (data not shown).

When we added 10⁻⁶ mol NA/l to the ganglion compartment (A₂ + NA) besides injecting 10 mg A₂/0.2 ml oil vehicle, 48 h before the incubation of the coeliac ganglion-SON-ovary system, the release of progesterone on day 19 increased significantly in relation to (A₂)s at all the studied times (**P < 0.05). On day 20, a decrease of progesterone was observed only at 120 min (0.09 ± 0.03 compared with 0.17 ± 0.03 ng/mg ovary, **P < 0.05), and on day 21, there was a significant increase of progesterone at 60 and 180 min (0.21 ± 0.03 compared with 0.09 ± 0.016 ng/mg ovary, **P < 0.05; 0.24 ± 0.04 compared with 0.13 ± 0.01 ng/mg ovary, **P < 0.01 respectively).

When we compared the experimental groups (A₂ + NA) in relation to the stimulation of coeliac ganglion with 10⁻⁶ mol NA/l, it was observed that the neural and endocrine joint action increased the release of progesterone on days 19 and 21 at all the studied times (#P < 0.05), and on day 20 only at 180 min (0.24 ± 0.03 compared with 0.10 ± 0.005 ng/mg ovary, #P < 0.01).

**Ganglionic effect of A₂ in the coeliac ganglion-SON-ovary system on the release of ovarian progesterone**

Figure 2A–C shows that the presence of 10⁻⁶ mol A₂/l in coeliac ganglion (A₂)g increased the release of progesterone in relation to the control at all the studied times on the days of pregnancy studied (days 19–21) (*P < 0.01).

The analysis of NA and A₂ joint action in the coeliac ganglion (NA + A₂)g shows that the release of ovarian progesterone tends to decrease in relation to the sole action of (A₂)g. On day 19 of pregnancy, the
inhibition of the release of progesterone was significant at 120 and 180 min (120 min: 0.32 ± 0.05 compared with 0.58 ± 0.06 ng/mg ovary, *P < 0.01; 180 min: 0.30 ± 0.03 compared with 0.55 ± 0.05 ng/mg ovary, *P < 0.01); on day 20, at 60 min (0.29 ± 0.03 compared with 0.40 ± 0.03 ng/mg ovary, *P < 0.05); and on day 21, at 120 min (0.15 ± 0.015 compared with 0.21 ± 0.016 ng/mg ovary, *P < 0.05).

Finally, when comparing the experimental groups (NA + A2) to the control, the release of progesterone did not exhibit significant changes on day 19, whereas on days 20 and 21, it increased significantly at all the studied times (#P < 0.01).

**Discussion**

As stated in the introduction, this work demonstrates that A2 might act through an endocrine pathway and, surprisingly, through a neural pathway, since it reverses the functional regression of the corpus luteum either systemically
or when added to the ganglion compartment. On the other hand, the NA and A₂ joint action is greater when A₂ is administered 48 h before incubation. Its luteotropic effect is likely to permit the rescue of the corpora lutea from regression. Consequently, NA action in the ganglion might be more effective.

Although A₂ is a weak androgen, it is the androgen of highest concentration in the circulation in rat (Gibori et al. 1988). Previous research has demonstrated that A₂ stimulates the luteal production of progesterone in rodents (Carrizo et al. 1994, Tellería et al. 1995, Thordarson et al. 1997). It has also been demonstrated that A₂ interferes with

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**Figure 2** Effect of the incubation of the coeliac ganglion-superior ovarian nerve-ovary system in the presence of NA + A₂ in the ganglion compartment on the release of ovarian progesterone. The work was carried out with rats on days 19 (A), 20 (B) and 21 (C) of pregnancy. When the coeliac ganglion-SON-ovary system is incubated with 10⁻⁶ mol A₂/l in the coeliac ganglion, the group is (A₂)g. When A₂ and NA, in 10⁻⁶ mol NA + A₂/l are added together on the coeliac ganglion, the group is considered (NA + A₂)g. Control (incubation of the coeliac ganglion-superior ovarian nerve-ovary system with buffer). NA (incubation of the coeliac ganglion-superior ovarian nerve-ovary system, with 10⁻⁶ mol NA/l alone in the ganglion compartment). Control and NA (Casais et al. 2001). Results are expressed as mean ± S.E.M. of six animals per experimental group (ANOVA I and Duncan test of multiple range) P < 0.05 and P < 0.01. (A₂)g versus control (*); (A₂)g versus (NA + A₂)g (•); (NA + A₂)g versus NA (#).
luteal regression by inhibiting apoptosis and stimulating progesterone production (Goyeneche et al. 2002).

A2 acts at two distinct levels: through the nuclear receptor expression (Goyeneche et al. 2002) and through a non-genomic mechanism, as it occurs in the human luteinized granulosa cells, in which this androgen specifically modifies the intracellular levels of calcium. This effect could not be either imitated with testosterone or blocked by androgen receptor blockers (Machelón et al. 1998).

When working with the coeliac ganglion-SON-ovary system of late-pregnancy rats with and without previous A2 injection 48 h before (systemic A2 effect), we observed that the presence of A2 in circulation increased the release of progesterone in the ovarian compartment, leading us to conclude that A2 shows trophic effect. This agrees with the previously mentioned findings.

Besides, as the coeliac ganglion-SON-ovary system offers the ability to analyse neuroendocrine effects (Sosa et al. 2000, 2004, Casais et al. 2001, Delgado et al. 2004), we incubated ex vivo coeliac ganglion-SON-ovary systems extracted from animals with previous A2 injection in the presence of NA in the ganglion compartment, and we determined the levels of ovarian progesterone release. The presence of catecholamine in the coeliac ganglion increased ovarian progesterone not only in relation to A2 injection but also when NA was added to the coeliac ganglion without previous A2 injection, leading us to suggest that A2 may prevent regression of the corpus luteum.

These results agree with our previous work (Casais et al. 2001) in which we demonstrated that the adrenergic agents in the coeliac ganglion assist gestation physiology; that is, they decrease the release of ovarian progesterone at the end of pregnancy. However, when A2 is present in the circulation, NA in the coeliac ganglion has an effect in late pregnancy (especially on day 21), indicating that androgen has kept the biosynthetic structure of progesterone at ovarian level. Yet, under these experimental conditions, it is not possible to determine whether A2 exerts its effects at ovarian or neural level.

There is an extensive literature about the action of gonadal steroids on the central nervous system (McEwen 1981, McEwen & Alves 1999, Woolley 1999, Hammond et al. 2001). However, few studies have been devoted to the action of gonadal steroids on the peripheral nervous system. Gejman and Cardinali (1983) suggest that muscarinic cholinergic neurotransmission in bovine and rat superior cervical ganglia may be affected by hormones.

In their review, Papka and Mowa (2003) show the presence of oestrogen receptors in spinal cord neurons and sensory and autonomic ganglionic neurons in sites that are associated with innervation of the female reproductive organs, and explain the role that oestrogen may play in these neurons and their circuits. On the other hand, Chen and Hua (1987) have shown the fast effect of glucocorticoid on neuronal membrane of hippocampal slices of rat and isolated coeliac ganglion of guinea pig. Hua and Chen (1989) also suggest that glucocorticoid can act non-genomically through its neuronal membrane receptor.

It is also known that the androgens can increase the volume, neuron number and synapses of developing rat superior cervical ganglion (Wright et al. 1991). Moreover, Félix et al. (2001) have shown an effect of testosterone on the electrical properties and nicotinic transmission of the major pelvic and coeliac ganglion neurons (nongenomic effect). The steroids are known to act through the steroid receptor and activate genomic pathways. Alternatives to the classical theory are theories of the non-genomic mechanisms, of which the hypothesis of membrane receptor-mediated effects in neurons is very important since rapid membrane effects form the basis of neuronal function.

Considering that a profuse fenestrate capilaris plexus has been described in the structure of prevertebral ganglia (Chau & Lu 1995), it is reasonable to suggest that A2 injected systemically reaches the ganglionic cells through the plexus. Aguado (2002) demonstrates that addition of serum to ganglion compartment in the coeliac ganglion-SON-ovary system increases the release of progesterone. These in vitro results support the idea that, in vivo, the humoral environment is highly influential on the functioning of the coeliac ganglion, thus affecting the ovary by a neural pathway.

When the direct ganglionic effect of A2 was analysed in the coeliac ganglion-SON-ovary system, the results were highly surprising. The levels of progesterone obtained by the application of A2 to the coeliac ganglion in relation to the controls were significantly higher on all the analysed days, and on day 21 they took longer (120–180 min) to reach the control levels of day 19. This would clearly demonstrate the neural action of the androgen in preventing the functional regression of the corpus luteum. We also compared the effect of androgen on the coeliac ganglion in relation to the addition of NA to the ganglion compartment (Casais et al. 2001), and we observed that the levels of release of progesterone with A2 in the coeliac ganglion were higher than those obtained with the addition of NA to the ganglion compartment on all the pregnancy days analysed. This action is more effective as the pregnancy age progresses. These facts support the idea that the steroid in the coeliac ganglion exerts a strong luteotropic effect differently from the catecholamines that play an important role in the physiology of pregnancy and the subsequent delivery (Casais et al. 2001). Finally, in the analysis of the neural and endocrine joint effect of NA and A2 on the ganglion, a constant significant decrease in the release of ovarian progesterone was observed compared with A2 alone. Although NA in the ganglion tends to diminish the luteotropic effect of A2, it is evident that the action of A2 prevails. Future study of apoptosis at ovarian level should be developed to explain the molecular mechanism of ganglionic A2 action.
Acknowledgements

This paper is dedicated to the memory of Luis I Aguado, PhD (1946-2003). We thank P Deis, PhD, who provided the progesterone and A2 antisera; C M Tellería, PhD, for critical revision of the manuscript; and Eng. Mario Baudino for his informative assistance. We also acknowledge the technical assistance of Luis Villegas. This work was supported by Grant 3711/92 from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina) and by Grant 9302 from Universidad Nacional de San Luis, Argentina. This work is part of the doctoral thesis of Marilina Casais. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received 21 June 2005
First decision 21 July 2005
Revised manuscript received 31 July 2005
Accepted 5 August 2005