Coeliac ganglion adrenergic activity modifies ovarian progesterone during pregnancy: its inter-relationship with LH

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Abstract

Most of the fibres that constitute the superior ovarian nerve (SON) originate at the neuronal bodies of the coeliac ganglion and innervate rat ovarian stroma cells. The purpose of this work was to study the part played by innervation on ovarian release of progesterone on day 15 and at the end of pregnancy in an integrated in vitro system known as the coeliac ganglion–SON–ovary system.

We also investigated, in the same system, whether there is some kind of inter-relationship between the effect of adrenergic agents and LH on progesterone release on day 15 of pregnancy.

The coeliac ganglion and the ovary were incubated in separate compartments, linked by the SON. The ovary was immersed in 2 ml buffer solution (ovarian compartment) and the coeliac ganglion was immersed in 2 ml of a different buffer solution (ganglion compartment). Under these conditions, the accumulation of progesterone in the ovarian compartment medium was used as an endpoint. Conditions were standardised on day 15 of pregnancy, when the decrease in the release of ovarian progesterone caused by non-specific stimulation on the ganglion with KCl (56 mM) demonstrated the functional integrity of the system. Neural influence was evaluated by the addition of adrenergic agents at a concentration of $10^{-5}$M to the coeliac ganglion. On day 15 of pregnancy, noradrenaline and propranolol increased progesterone release while phentolamine diminished it. The existence of ganglionic tone was assessed by analysing progesterone basal levels at different stages of pregnancy. The highest secretion of progesterone was found to take place on day 15, diminishing as pregnancy advanced. In addition, adrenergic neural participation was studied during the physiological luteolysis occurring at the end of pregnancy. Major findings were that noradrenaline increased ovarian accumulation of progesterone on day 19 and decreased it on day 20, while propranolol and phentolamine diminished progesterone release on both days. In additional studies, some neuroendocrine aspects were investigated at a peripheral level. The addition of LH only to the ovarian compartment did not affect progesterone secretion. However, when LH in the ovarian compartment was accompanied by noradrenaline, propranolol or phentolamine in the ganglion compartment, the release of progesterone decreased.

It can be concluded that modifications of the neural state of the coeliac ganglion affect ovarian progesterone secretion and the physiology of pregnancy via the SON. The results may confirm that the coeliac ganglion–SON–ovary system provides a direct link between the autonomic nervous system and physiological events during pregnancy.

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Introduction

Most of the fibres of the superior ovarian nerve (SON) come from the postganglionic sympathetic neurones of the coeliac ganglion and innervate ovarian stroma cells (Lawrence & Burden 1980, Klein & Burden 1988). The ovarian cell membranes have adrenergic receptors and their occupation with adrenergic agents modifies the release of ovarian steroids. This fact shows the participation of the peripheral nervous system in ovarian function (Condon & Black 1976, Coleman et al. 1979, Harwood et al. 1980, Adashi & Hsueh 1981, Aguado et al. 1982, Aguado & Ojeda 1984a,b, Norjavaara et al. 1984, 1989, Sosa et al. 2000). Furthermore, some neurotransmitters that stimulate ovarian progesterone release such as noradrenaline and vasoactive intestinal peptide (Ojeda & Aguado 1985, Dees et al. 1986, Ojeda & Lara 1989, Dissen et al. 1993, Gerendai et al. 1995, Kalekzyc et al.1995), or peptides with inhibitory effects such as gonadotrophin-releasing hormone (Hsueh & Erickson 1979, Huesh & Jones 1981, Pieper et al. 1981, Sheela Rani et al. 1983) and gamma aminobutyric acid (Erdő et al. 1985, Häppölä et al. 1987) have been found in the ovary. Some of them are also present in the SON and the coeliac ganglia (Jan & Jan...
also proved a local relationship in the ovary between noradrenaline and LH in in vitro systems of isolated cells (Harwood et al. 1980, Jena & Abramowitz 1989).

Materials and Methods

Animals

Pregnant rats bred in our laboratory and originally of the Holtzman strain were used. The rats were kept under controlled conditions, with lights on from 0700 to 1900 h, and at a temperature of 24 ± 2 °C, with free access to standard rat chow (Cargil SACI; Saladillo, Buenos Aires, Argentina) and water. Vaginal smears were taken daily and only rats with 4-day oestrous cycles were used. Female rats were caged individually with fertile males on the night of the day of pro-oestrus, and the presence of spermatozoa was checked in the vaginal smear the following morning. This day was designated as day 0 of pregnancy. Our colony usually give birth on day 22.

Groups of six to eight animals on days 15, 19, 20 and 21 of pregnancy were used for each experimental procedure. The experiments were performed in accordance with the Guidelines on the Handling and Training of Laboratory Animals, The Biological Council, UFAW 1992.

Reagents

The following drugs were purchased from Sigma Chemical Co. (St Louis, MO, USA): l-isoproterenol hydrochloride (Iso), 1-d-norepinephrine hydrochloride (NE), l-propranolol hydrochloride (Prop), phenolamine hydrochloride (Ph), HEPES, medium 199, EDTA, collagenase type IV (570 U/mg), dextrose, ascorbic acid, bovine serum albumin fraction V (BSA) and LH. 1,2,6,7−[3H]Progesterone was provided by New England Nuclear (Boston, MA, USA). Other reagents were of analytical grade. Luteal cells were incubated in 24-well plastic tissue culture dishes (Corning Laboratory Sciences Co., Cambridge, MA, USA).

Surgical procedure and characterisation of the coeliac ganglion–SON–ovary system

The surgical procedure used for removing the system, its characterisation and histological control, as well as standardisation of the incubation times were performed as described previously (Sosa et al. 2000). The system was removed by dissection, avoiding contact between the surgical instruments and the nerve fibres in order to prevent spontaneous depolarisation of the nerves. The piece of tissue removed consisted of the left ovary, the fibres that constitute the SON, inserted in the suspensory ligament, and the coeliac ganglion accompanied by some small ganglia that surround it. The total surgical procedure...
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Figure 1 Schematic representation of the cuvette with two compartments for the incubation of the coeliac ganglion–SON–ovary system. CG, coeliac ganglion; O, ovary; P, progesterone.

was completed in 1–2 min. In order to verify the existence of the ganglion, routine histological techniques were followed throughout the characterisation of the system.

Once the coeliac ganglion–SON–ovary system was removed, it was washed with incubation medium and placed in a cuvette with two compartments. Each compartment contained 2 ml Krebs–Ringer bicarbonate buffer (pH 7.4), with the addition of glucose (0.1 mg/ml) and albumin (0.1 mg/ml), as has been described for the incubation of ovaries in other in vitro systems (Aguado et al. 1982, Lara et al. 1990a,b, Ferruz et al. 1992).

The coeliac ganglion and the ovary were placed in two separate compartments connected by the SON (Fig. 1). The system was stabilised by preincubation in a metabolic bath at 37 °C for 30 min in an atmosphere composed of 95% O₂ and 5% CO₂. The end of the preincubation period was considered as incubation time 0. At this time, the buffer was changed in both compartments, and periodical extractions were made from the ovarian compartment at 30, 60, 120 and 180 min for determination of the release of progesterone. Ascorbic acid (0.1 mg/ml in Krebs–Ringer) was added as an antioxidant agent to the ganglion compartment at incubation time 0 (Dhariwal et al. 1989, Behrman et al. 1996).

Experimental procedure

Rats on days 15, 19, 20 and 21 of pregnancy were used. They were anaesthetised with ether and the system was removed by surgery. The fetuses had been previously removed and were killed in an atmosphere of ether. The surgical procedure was performed between 0900 and 1000 h. The coeliac ganglion–SON–ovary system was removed and placed in the above-described cuvette, taking care that the SON was kept moist with the work solution. The values of the progesterone released under these conditions were considered to be the control (control group). For the experimental groups, the non-specific and specific agents to be tested were added to the ganglion compartment and progesterone release was measured. KCl (56 mM) was used as a non-specific depolarising stimulus (Koh & Hille 1997). The adrenergic agents used were NE as adrenergic agonist, Prop as β antagonist and Ph as α antagonist. The different substances were dissolved in equal concentrations (10⁻⁶ M) and volumes (2 ml) of Krebs–Ringer solution plus ascorbic acid.

The samples of liquid from the ovarian compartment were maintained at -20 °C until determination of progesterone by radioimmunoassay (RIA). The results are expressed as ng progesterone/mg ovarian tissue against the time of incubation. Corresponding corrections were made in all cases, taking into consideration the volume extracted in each test period.

Luteal cell dispersion of ovaries from pregnant rats

Luteal cells from pregnant rats were isolated as described previously (Carrizo et al. 1994, Tellería et al. 1994). The procedure was performed between 0900 and 1000 h. Briefly, ovaries from rats decapitated on day 15 of pregnancy were collected and placed in phosphate-buffered saline (PBS; pH 7.4). The corpora lutea were dissected and luteal cells were dispersed and incubated in PBS, in the presence of collagenase (1 mg/ml) and BSA (1%) for 1 h at 37 °C in a stirred waterbath (100 r.p.m.). The dispersed cells were separated by centrifugation for 5 min at 800 g. The luteal cells were washed in PBS–EDTA (1 mM) and resuspended in medium 199 containing HEPES (10 mM). Cell incubation was performed at a final concentration of 3 × 10⁵ viable cells/ml in medium 199 at 37 °C under an atmosphere of 95% air and 5% CO₂ for 4 h.

Iso was dissolved in medium 199 and added in a volume of 10 µl to make a final concentration of 10⁻⁶ M. At the end of the incubation period, cells were harvested and the media were frozen at -20 °C until the measurement of the progesterone content by RIA.

The results are expressed as progesterone (ng/ml per 3 × 10⁵ cells per 4 h).

Coeliac ganglion–SON–ovary system incubations with LH

The coeliac ganglion–SON–ovary system was also used to study some neuroendocrine aspects at peripheral levels. The values of progesterone release obtained when ovine LH was added (50 ng/ml in incubation buffer) to the ovarian compartment and incubation medium plus ascorbic acid to the ganglion compartment were considered to be basal (control-LH group). Those groups in which LH was not present in the ovary were considered to be controls. The experimental groups were those in which LH was present in the ovarian compartment and the adrenergic agents (NE, Prop or Ph) in the ganglion compartment. The levels of progesterone released in the ovarian compartment were determined by RIA.
**Progesterone assay**

Progesterone was measured by RIA using antiserum 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 has been reported previously (Bussmann & Deis 1979, Tellería et al. 1994). The sensitivity of the assay was less than 5 ng/ml serum and the inter and intra-assay coefficients of variation were less than 10%. This assay has been validated previously (Bussmann & Deis 1979, Donoso 1988).

**Statistical analysis**

Results are presented as means ± S.E.M. in each group of six to eight rats. Differences between two groups were analysed with Student’s t-test. Analysis of 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**

**Coeliac ganglion–SON–ovary system: effect of the addition of KCl to the ganglion compartment on progesterone release by the ovary on day 15 of pregnancy**

KCl is considered to be a non-specific stimulating agent on the preganglionic nerve fibres. When KCl (56 mM) was added to the ganglion compartment, the progesterone released by the ovary significantly decreased in comparison with the control at all times studied (Fig. 2).

**Effect of the addition of adrenergic agents to the ganglion compartment on ovarian release of progesterone on day 15 of pregnancy**

The addition of adrenergic agents on the coeliac ganglion led to significant variations in ovarian progesterone release at all times studied. When the stimulation was carried out with NE or Prop, progesterone secretion increased significantly as compared with the control ($P<0.01$ and $P<0.05$ respectively), while Ph induced a significant decrease in the release of progesterone, compared with the control ($P<0.05$) (Fig. 3).

**Effect of Iso on progesterone secretion by incubated luteal cells**

Luteal cells from the corpus luteum of rats on day 15 of pregnancy were incubated. The incubation was performed in the absence (control) and the presence of Iso (control+Iso), at a final concentration of $10^{-6}$M, in the incubation medium. Figure 4 shows that the presence of the adrenergic agonist did not modify the progesterone release from incubated luteal cells in relation to the control values.
Control means are the means * of quadruplicate determinations of three different experiments. NS, not significant compared with the control group (Student’s *t*-test).

Figure 4  Progesterone (P) production by luteal cells from 15-day pregnant rats in the absence (Control) or presence of Iso (10−6M; Control+Iso) in the incubation medium. Cells were incubated at 37°C under an atmosphere of 95% O2–5% CO2 for 4 h. Values are the means ± S.E.M. of quadruplicate determinations of three different experiments. NS, not significant compared with the control group (Student’s *t*-test).

Figure 5  Progesterone (P) release by the ovary in the coeliac ganglion–SON–ovary system obtained from rats on days 15, 19, 20 and 21 of pregnancy. The systems were incubated in Krebs–Ringer solution plus ascorbic acid (0·1 mg/ml in Krebs–Ringer) in the ganglion compartment at 37°C in an atmosphere of 95% O2–5% CO2 for 180 min. Values are the means ± S.E.M. from six to eight animals per experimental group.

Study of the existence of autonomic ganglionic tone in the coeliac ganglion–SON–ovary system

To verify that the coeliac ganglion–SON–ovary system is a functional entity with its own autonomic tone, the control values of progesterone release on the different days of pregnancy studied (Fig. 5) were analysed. The highest values were those on day 15 of pregnancy and, in comparison with this day, the levels diminished as the end of pregnancy approached. On day 19, only one significant decrease in relation to day 15 was observed, at 120 min of incubation (0·22 ± 0·04 compared with 0·32 ± 0·03 ng/mg ovary, *P*<0·05), whereas on days 20 and 21 a significant decrease compared with day 15 was observed, at all times studied (*P*<0·01).

Effect of the addition of adrenergic agents to the ganglion compartment on release of ovarian progesterone at the end of pregnancy

The same coeliac ganglion–SON–ovary experimental scheme used on day 15 was used to investigate whether the adrenergic action on the release of ovarian progesterone was still significant at the end of pregnancy. The results obtained showed that on day 19 NE increased the release of progesterone at all times studied in relation to the control (*P*<0·05). Prop caused a significant decrease at 30 and 120 min (0·10 ± 0·02 compared with 0·21 ± 0·04 ng/mg ovary, *P*<0·01 and 0·15 ± 0·03 compared with 0·22 ± 0·04 ng/mg ovary, *P*<0·05) while Ph decreased progesterone release at all times studied compared with the control (*P*<0·01) (Fig. 6A).

On day 20, the three agents studied significantly decreased the levels of progesterone at all times studied (Fig. 6B), whereas on day 21 no significant differences were observed as compared with the control (Fig. 6C).

Effect of the addition of LH to the ovarian compartment on the release of progesterone on day 15 of pregnancy

The presence of the gonadotrophin in the ovarian compartment did not modify the release of progesterone in relation to the control at any of the times studied (Fig. 7). All the adrenergic agents studied caused a decrease in the ovarian response as compared with control-LH when they were added in the ganglion compartment and LH was added in the ovarian compartment at all times studied (Fig. 8). This decrease was also observed when the presence of NE or Prop in the ganglionic compartment together with LH in the ovarian compartment was compared with the sole stimulation of either NE or Prop in the coeliac ganglion. On the other hand, Ph maintained its inhibitory effect with and without LH. The above-described results are compared in Table 1, in which the results of Figs 3, 7 and 8 have been repeated for clarity.

Discussion

Studies on the participation of the SON in ovarian function have been performed on prepuberal, peripuberal, cyclic adult and pseudopregnant rats (Condon & Black 1976, Harwood et al. 1980, Adashi & Hsueh 1981, Jordan 1981, Aguado et al. 1982, Aguado & Ojeda 1984a,b, Norjavaara et al. 1984, 1989, Sosa et al. 2000). However, little attention has been paid to this issue in pregnant rats (Burden 1985). It is to be noted that progesterone release
has been shown to be one of the processes most sensitive to neural regulation (Aguado et al. 1982). The corpus luteum is the main source of progesterone (Gibori 1993) and so we have studied the neural effect on the ovary during pregnancy, where the corpus luteum is the predominant structure.

Figure 6 Effect of agonist and antagonist adrenergic agents in the ganglion on ovarian progesterone (P) release in the coeliac ganglion–SON–ovary system obtained from rats on days 19 (A), 20 (B) and 21 (C) of pregnancy. The systems were incubated in Krebs–Ringer solution at 37 °C in an atmosphere of 95% O₂–5% CO₂ for 180 min. Ascorbic acid (0.1 mg/ml in Krebs–Ringer) without (Control) and with adrenergic agents at a concentration of 10⁻⁶ M was added to the ganglion compartment. Values are the means ± S.E.M. from six to eight animals per experimental group. *P<0.01, •P<0.05 compared with the respective control groups (ANOVA I–Duncan’s multiple range test).

Figure 7 Progesterone (P) release by the ovary in the coeliac ganglion–SON–ovary system obtained from rats on day 15 of pregnancy with or without LH (50 ng/ml) in the ovarian compartment. The system was incubated in Krebs–Ringer solution, plus ascorbic acid in the ganglionic compartment, at 37 °C in an atmosphere of 95% O₂–5% CO₂ for 180 min and in the ovarian compartment in the absence (Control) or presence of LH (Control-LH). Values are the means ± S.E.M. from six to eight animals per experimental group. NS, not significant compared with the control group (Student’s t-test).

Figure 8 Effect of adrenergic agents in the ganglion and LH (50 ng/ml) in the ovary on the release of ovarian progesterone (P), using the coeliac ganglion–SON–ovary system obtained from rats on day 15 of pregnancy. The system was incubated in Krebs–Ringer solution at 37 °C in an atmosphere of 95% O₂–5% CO₂ for 180 min. LH was added to the ovarian compartment and ascorbic acid (0.1 mg/ml in Krebs–Ringer) without (Control-LH) and with adrenergic agents (10⁻⁶ M) added to the ganglion compartment. Values are the means ± S.E.M. from six to eight animals per experimental group. *P<0.01, •P<0.05 compared with the Control-LH group (ANOVA I–Duncan’s multiple range test).
Table 1 Progesterone release by the ovary at different times in a coeliac ganglion–SON–ovary system obtained from rats on day 15 of pregnancy. The system was incubated in Krebs–Ringer solution plus ascorbic acid in the ganglionic compartment, at 37°C in an atmosphere of 95% O₂–5% CO₂ for 180 min, in the absence (Control) or presence of LH (50 ng/ml) (Control-LH) in the ovarian compartment. The system was also incubated with ascorbic acid (0·1 mg/ml in Krebs–Ringer) and adrenergic agents (10⁻⁶ M) in the ganglionic compartment and without LH in the ovarian compartment (NE or Prop or Ph). The system was also incubated with ascorbic acid (0·1 mg/ml in Krebs–Ringer) and adrenergic agents (10⁻⁶ M) in the ganglionic compartment and with LH in the ovarian compartment. Values are the means ± s.e.m. from six to eight animals per experimental group.

<table>
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<th>Time (min)</th>
<th>Control</th>
<th>Control-LH</th>
<th>NE</th>
<th>LH-NE</th>
<th>Prop</th>
<th>LH-Prop</th>
<th>Ph</th>
<th>LH-Ph</th>
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<tr>
<td>30</td>
<td>0·20 ± 0·017</td>
<td>0·29 ± 0·03</td>
<td>0·48 ± 0·03</td>
<td>0·16 ± 0·02*</td>
<td>0·33 ± 0·024</td>
<td>0·24 ± 0·04*</td>
<td>0·1 ± 0·02</td>
<td>0·13 ± 0·02NS</td>
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<tr>
<td>60</td>
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<td>0·32 ± 0·02</td>
<td>0·72 ± 0·096</td>
<td>0·19 ± 0·03*</td>
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*P<0·01, NE compared with NE-LH; P<0·01, Prop compared with Prop-LH; NS, not significant, Ph compared with Ph-LH at all times analysed (ANOVA–Duncan’s multiple range test). Other statistical comparisons are made in Figs 3, 7 and 8.

The present study provides evidence that the response of the ovary to a neural stimulus varies in the different stages of pregnancy studied. Additionally, the results indicate that there is an inter-relationship between the adrenergic ganglionic effect and the endocrine action of LH on the ovary. These results were obtained using the coeliac ganglion–SON–ovary system previously standardised in our laboratory for the oestrous cycle (Sosa et al. 2000). The system characterisation was performed on day 15 of pregnancy, since this is the time of maximum activity of the corpus luteum (Uchida 2000) and therefore it was assumed that the sensitivity of the system to neural inputs would be increased on this day. Addition of a moderate concentration (56 mM) of KCl (Koh & Hille 1997) to the ganglionic compartment on this day caused a significant decrease in progesterone release at all times, in comparison with the basal level. Since KCl is a non-specific depolarising agent of ganglionic cells, it is very probable that the release of several neurotransmitters was favoured, thus leading to an inhibitory effect on ovarian function. The ovarian response observed demonstrated the functional integrity of the coeliac ganglion–SON–ovary system. In addition, the ganglion did not exhibit microscopic changes when studied at the end of each experiment, which also supports the viability of the system.

Even though acetylcholine is a typical preganglionic sympathetic neurotransmitter (Sarper 1995), the coeliac ganglion was stimulated with adrenergic agents once the characterisation had been performed. This choice was based on the fact that the coeliac ganglion is innervated by fibres of an adrenergic nature which come from the medulla and by other preaortic ganglia (Sarper et al. 1976, Messenger & Furness 1992). In addition, it is to be noted that α and β adrenergic receptors have been detected in the superior cervical ganglion and other ganglia (Pinto et al. 1991, Shivachar & Eikenburg 1999). The effect of adrenergic ganglionic stimulation on ovarian progesterone release has also been demonstrated in our laboratory using the same integrated system (Sosa et al. 2000).

The addition of NE to the ganglion compartment on day 15 of pregnancy showed a strong increase in the release of progesterone from the ovary. Progesterone secretion was also stimulated by Prop while Ph diminished it significantly. When luteal cells from corpora lutea of rats on day 15 of pregnancy were incubated in the presence of 10⁻⁶ M Iso, an adrenergic β agonist, no variations were observed in progesterone production. This demonstrates poor sensitivity of luteal cells to direct adrenergic stimulation, in spite of the presence of β receptors in the membranes of the ovarian cells (Harwood et al. 1979).

The differences observed in the results obtained with the two experimental schemes used demonstrates that the integrity of the system is necessary for ovarian response to neural action. This indicates that the system used here has the advantages of preserving intraovarian factors and keeping SON innervation intact.

On the other hand, the existence of ganglionic tone under our experimental conditions was confirmed by analysing the variations of the basal levels of progesterone in the different stages of pregnancy studied. The highest values corresponded to day 15 of pregnancy and, as expected, progesterone release decreased as pregnancy advanced. The ganglionic tone allows the use of antagonists in the ganglion without simultaneous addition of the corresponding agonist.

Having demonstrated the action of innervation on day 15 of pregnancy, the same coeliac ganglion–SON–ovary system was used to analyse its action during the physiological luteolysis occurring at the end of pregnancy on days 19, 20 and 21 (Hurwitz & Adashi 1993). On day 19, NE added to the ganglion compartment significantly increased progesterone release while Prop and Ph decreased it. It might be inferred that the effect of NE on progesterone
release would take place through α or β adrenergic receptors, but our experimental scheme does not allow us to discern whether they are pre- or post-ganglionic. The interpretation of these results is further complicated by the presence of interneurones with peptidergic and dopaminergic neurotransmitters, among others (Mentel et al. 1976, Polonyi et al. 1982, Matthews 1989).

On day 20, a significant reduction of progesterone was observed with both NE and its antagonists, while on day 21 no significant effects were detected with any of the adrenergic agents studied. This therefore suggests that the steroidogenic response to neural action gradually decreases with the establishment of physiological luteolysis at the end of gestation (Hurwitz & Adashi 1993).

In the present work, some neuroendocrine aspects were also studied at the peripheral level. Other authors have observed an inter-relationship between catecholamines and LH in cultures of luteal cells of rats at other physiological stages (Harwood et al. 1980, Jena & Abramowitz 1989).

The addition of LH to the ovary on day 15 of pregnancy in the system under study did not lead to any variations in the release of progesterone. This was to be expected, since LH levels exhibit very low values on day 15 (Morishige et al. 1973, Taya & Greenwald 1981). However, when the addition of LH to the ovary was accompanied by the addition of NE, Prop or Ph to the ganglionic compartment, progesterone production fell. From Table 1 it can be inferred that addition of LH to the ovary decreases the augmented progesterone release provoked by the addition of NE or Prop to the ganglion, while it does not modify the ovarian response provoked by the addition of Ph to the ganglion.

Overall, it can be concluded that the coeliac ganglion–SON–ovary system used here provides a good resemblance of in vivo conditions, particularly of ovarian innervation. As shown, at the time of the highest production of progesterone (day 15 of pregnancy), NE produces a noticeable increase of the analysed steroid, which supports its role in the maintenance of pregnancy. This neural influence is maintained until day 19 and decreases as the end of pregnancy approaches, helping the conditions of birth. In other words, the neural action adds up to the other factors involved in the reproductive phenomenon. The results obtained here may represent an advance in the knowledge of the role of neural control in the ovary’s function during pregnancy. Furthermore, they might help to further understand certain pathologic states of reproduction that cannot be explained by purely hormonal causes.

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