UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE ESTUDIOS SUPERIORES "ZARAGOZA"

BIOLOGÍA EXPERIMENTAL

INTERACCIÓN DE LA INERVACIÓN NORADRENÉRGICA Y VIPÉRGICA EN LA RATA ADULTA CON O SIN EL SÍNDROME DEL OVARIO POLIQUÍSTICO

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

GABRIELA ROSAS GAVILÁN

TUTOR PRINCIPAL DE TESIS: Dr. ROBERTO DOMÍNGUEZ CASALÁ
Facultad de Estudios Superiores Zaragoza, UNAM.

COMITÉ TUTOR: Dra. LETICIA MORALES LEDESMA
Facultad de Estudios Superiores Zaragoza, UNAM.
Dr. PABLO GUSTAVO DAMIÁN MATZUMURA
Universidad Autónoma Metropolitana, Unidad Iztapalapa.

MÉXICO, D.F. DICIEMBRE, 2015.

UNAM
POSGRADO
Ciencias Biológicas

COORDINACIÓN

Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a Usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 28 de septiembre de 2015, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** del (la) alumno (a) **GABRIELA ROSAS GAVILÁN** con número de cuenta **401052578** con la tesis titulada **"Interacción de la inervación noradrenérgica y VIPérgica en la rata adulta con o sin el Síndrome del Ovario Poliquístico"** realizada bajo la dirección del **DR. ROBERTO DOMÍNGUEZ CASALÁ**:

Presidente:	DR. JOSÉ MIGUEL BETANCOURT RULE
Vocal:	DRA. MARÍA ESTHER CRUZ BELTRÁN
Secretario:	DR. PABLO GUSTAVO DAMIÁN MATZUMURA
Suplente:	DRA. MARCELA ARTEAGA SILVA
Suplente:	DRA. LETICIA MORALES LEDESMA

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F., a 29 de octubre de 2015.

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA
COORDINADORA DEL PROGRAMA

c.c.p. Expediente del (la) interesado (a)

Gabriela Rosas Gavilán

AGRADECIMIENTOS

Al **Posgrado en Ciencias Biológicas de la UNAM** por darme la oportunidad de entrar en su programa y adquirir nuevos conocimientos que me han permitido fortalecer mi formación académica y profesional.

Al **Consejo Nacional de Ciencia y Tecnología** por el apoyo económico otorgado para realizar mis estudios de doctorado por medio de la beca número 29187, y al apoyo financiero otorgado por **DGAPA-PAPIIT** convenio IN-211813.

A mi tutor principal y a los miembros del comité tutor:

Dr. Roberto Domínguez Casalá.

Dra. Leticia Morales Ledesma.

Dr. Pablo Gustavo Damián Matzumura.

Por dedicar su valioso tiempo a la revisión de este estudio y compartir sus conocimientos para enriquecerlo.

A mi querida universidad, la **UNAM**, y a mi entrañable facultad, **FES-Zaragoza**, porque bajo la dirección de grandes académicos contribuyó a fortalecer mi formación profesional. Gracias por el orgullo de ser sangre azul, piel dorada.

A los **Miembros del Jurado:**

Dr. José Miguel Betancourt Rule

Dra. María Esther Cruz Beltrán

Dr. Pablo Gustavo Damián Matzumura

Dra. Marcela Arteaga Silva

Dra. Leticia Morales Ledesma

Por el tiempo dedicado en la revisión de este estudio, al que enriquecieron con sus conocimientos y con sus valiosas observaciones.

A la **Dra. Leticia Morales Ledesma**, le agradezco infinitamente el haberme enseñado a ser autosuficiente y crítica, por que cada uno de sus consejos y palabras de aliento me confortaron y ayudaron a superarme cada día, pero sobre todo le agradezco por su valiosa amistad que me ha brindado a lo largo de tantos años. Gracias doctora Lety por alentarme a cada minuto a concluir mis estudios de posgrado.

Al **Dr. Roberto Domínguez Casalá**, por dirigir de manera exigente y objetiva esta tesis, por compartir conmigo sus conocimientos, por ayudarme a corregir mis errores, por sus valiosos consejos, su inagotable paciencia, su invaluable amistad y por buscar siempre mi crecimiento profesional... gracias doctor.

Al **Dr. Pablo Gustavo Damián Matzumura**, por aceptar ser parte de mi comité tutor, seguir de cerca mi desarrollo académico, enriquecer este estudio con sus observaciones y haber contribuido no solo en mi formación profesional sino también personal. Gracias doctor por su apoyo, sus consejos y por haberme brindado su amistad.

A mis niñas, **Isa, Liz y Chío** porque en estos cuatro años cada una de ustedes fue parte importante de este proyecto. Les agradezco que hayan formado parte de mi vida y me hayan brindado su amistad… aprendí cosas distintas de cada una de ustedes. Quiero que sepan que siempre traté de explicarles, ayudarlas y orientarlas lo mejor posible. Les deseo lo mejor, hoy y siempre, que nunca desistan y que logren el mayor de los éxitos. Las quiero mucho niñas.

A **Rosa, Dey y Ely**, por su cariño y amistad, por todo los consejos y el apoyo que me brindaron durante el tiempo que hemos compartido. Gracias chicas, porque sin ustedes esta etapa de mi vida no hubiera sido la misma ¡¡las quiero mucho!!.

A cada uno de mis compañeros y amigos del *laboratorio 7 de la Unidad de Investigación en Biología de la Reproducción:* Rosse, Dey, Ely, Azu, Ricardo, Isa Ramírez, Liz, Chío, Isa Navarrete, Noesito, Alina, Aldo, Iván, Hazael, Dani, Julio, Cata, David, Naye, Wendy, Misael y Valeria… gracias por hacer de mi estancia en el laboratorio un lugar muy especial.

A la **Dra. Paty** y a la **Dra. Esther** por compartir conmigo sus conocimientos y su valiosa amistad.

A la Dra. Adriana, a la Dra. Ely, al Dr. Román y al personal técnico del Bioterio de la FES-Zaragoza, por su apoyo en el cuidado de los animales utilizados en este estudio y por su amistad.

Al Biol. Roberto Chavira por su colaboración en la cuantificación de hormonas esteroides.

DEDICATORIAS

A ti, mi *Diosito:*

Por acompañarme día a día, por nunca alejarme de tu mano, por darme la fortuna de tener a mi familia completa, por guiarme siempre por el mejor camino, por no dejarme desistir y por permitirme culminar este gran sueño… mi doctorado. Gracias Diosito, te amo.

A mis Papás, *Ger y Lupita:*

Por ser mí mejor ejemplo a seguir y por la fortuna ser su hija. A mi mami, por su amor y ternura, a mi Pa, por su guía y mano firme, porque los dos lograron darme el equilibrio que necesité y hacer de mí una persona responsable y perseverante. Gracias por el amor incondicional y la confianza que siempre me han brindado, por ser mis pilares y darme la fortaleza para seguir adelante y por fundar en mí el espíritu de superación. Les agradezco infinitamente cada palabra de aliento, sus sabios consejos y su incansable paciencia, sin ustedes no hubiera logrado concluir esta meta. Gracias por todo, los amo infinitamente.

A mi niña, *Jaque:*

Porque hace 17 años llegaste a iluminar mi vida y desde entonces llenaste mi mundo de alegría, cariño y esperanza. Gracias nena por hacer de mi vida algo especial y maravilloso, por tus palabras de aliento en los momentos difíciles, por acompañarme en todo momento y por permitirme ser parte importante en tu vida. Quiero que sepas que eres mi tesoro más preciado, la razón para superarme día a día y mi más grande proyecto. Espero que tu vida este llena de muchos amigos, amores y éxitos. Te amo chapis.

A mis hermanos, *Dani y César:*

Por todos los momentos que hemos pasado juntos, por el apoyo que me han brindado, por compartir conmigo tantas risas, alegrías y tristezas, y porque aún cuando nuestras vidas cambien y nuestras prioridades también, nosotros sigamos siempre unidos. Gracias por ser mis mejores amigos, los quiero mucho compadritos, nunca lo olviden.

A *papá Alfonso:*

Por todo el cariño que me diste, porque hiciste de mi niñez algo hermoso, por todos esos lindos recuerdos que tengo de ti y porque hoy, después de tanto tiempo que ha pasado desde que nos dejaste, tu paso por mi vida sigue aún fresco y porque a través de mis ojos, mi niña a aprendido a quererte. Te amo Papá Alfonso y te extraño mucho.

A mis sobrinitas, *Fanny y Yamy:*

Por ser esos pequeños angelitos que con su cariño, sus sonrisas, su ternura y sus travesuras han llenado nuevamente de alegría la casa. Las quiero mucho nenitas y les deseo lo mejor en esta vida.

A *Rosse:*

Gracias amiga por tu amistad, por todo tu cariño, porque desde la licenciatura te convertiste en una parte importante de mi vida y porque hoy después de tantas experiencias vividas, sueños compartidos, alegrías y tristezas, aún seguimos juntas, compartiendo nuestro día a día, te quiero mucho amiga y espero que nuestra amistad siga creciendo.

ESTRUCTURA DE LA TESIS

En esta tesis se analizaron los efectos agudos de la estimulación VIPérgica de los ovarios de animales cíclicos o con síndrome del ovario poliquístico sobre la secreción de hormonas esteroides, y si tales efectos dependen de las señales nerviosas del nervio ovárico superior. La información se agrupó en secciones generales y en capítulos con temas específicos. La primera parte de esta tesis corresponde a la introducción general, mientras que el resto a los manuscritos donde se describen y analizan los resultados de los estudios realizados y que han sido publicados o enviados a revistas para su publicación. Los manuscritos atienden las normas editoriales de cada revista.

El capítulo I incluye las bases teóricas que sustentan esta investigación. En el capítulo II se muestran los efectos agudos (1 hora) y subagudos (24 horas) de la inyección unilateral de VIP en la bursa ovárica de animales en cada día del ciclo estral. Estos resultados fueron publicados en la revista Endocrine (2015) 48: 968-977. En el capítulo III se abordan los efectos de la estimulación VIPérgica del ovario inervado o denervado de animales cíclicos con sección unilateral del NOS. En el capítulo IV se analizan los efectos agudos de la inyección de VIP en la bursa ovárica de ratas con SOPQ que fueron o no sometidas a la sección unilateral del NOS. Finalmente, en la discusión general se analiza el posible significado de los resultados más relevantes obtenidos en el presente estudio.

ÍNDICE DE CONTENIDO

Páginas

ABREVIATURAS

20α-HSD. 20α-hidroxiesteroide deshidrogenasa

3β-HSD. 3β-hidroxiesteroide deshidrogenasa

AE-PCOS. Sociedad del SOPQ y Exceso de Andrógenos

AMPc. Adenosín monofosfato cíclico

BE. Benzoato de estradiol

DHEA. Dehidroepiandrosterona

DHT. Dihidrotestosterona

FSH. Hormona estimulante del folículo

GCMS. Ganglio Celiaco-Mesentérico Superior

GnRH. Hormona liberadora de Gonadotropinas

GTD. Guanetidina

IGF-I. Factor de Crecimiento Semejante a la Insulina-I

Kit-L. Ligando Kit

LH. Hormona Luteinizante

NA. Noradrenalina

NGF. Factor de Crecimiento Neural

NICHD. Instituto Nacional de Salud Infantil y Desarrollo Humano

NOS. Nervio Ovárico Superior

NPO. Nervio del Plexo Ovárico

NPY. Neuropéptido Y

NV. Nervio Vago

OPQ. Ovarios Poliquísticos

PKA. Proteína cinasa A

PT. Propionato de Testosterona

SOPQ. Síndrome del Ovario Poliquístico

StAR. Proteína reguladora de la esteroidogénesis aguda

TH. Tirosina Hidroxilasa

tPA. Activador tisular del plasminógeno

VE. Valerato de Estradiol

VIP. Péptido Intestinal Vasoactivo

RESUMEN

Las funciones ováricas son reguladas por señales endocrinas, inmunológicas y nerviosas de naturaleza simpática, sensorial y parasimpática. El nervio ovárico superior (NOS) es la principal fuente de inervación simpática del ovario por el que transcurren fibras que contienen neuropéptido Y (NPY), noradrenalina (NA) y péptido intestinal vasoactivo (VIP).

Estudios *in vitro* muestran que en células de la granulosa y en el ovario completo de la rata, la NA estimula la secreción de progesterona, androstenediona y testosterona, mientras que el VIP incrementa la secreción de progesterona, andrógenos y estradiol. En ratas con síndrome del ovario poliquístico (SOPQ) inducido por la inyección de valerato de estradiol (VE), la actividad de las fibras del NOS es mayor que en el grupo control, lo que resulta en una mayor concentración de NA y VIP en el ovario. En cultivo de ovarios de ratas con SOPQ, el agregado de NA al medio de cultivo incrementa la liberación de progesterona y andrógenos, mientras que el agregado de VIP aumenta la secreción de andrógenos y estradiol. En ratas con SOPQ, la sección unilateral o bilateral del NOS normaliza las concentraciones de testosterona y estradiol en suero.

El objetivo del presente estudio fue analizar los efectos agudos de la inyección unilateral de VIP en la bursa ovárica de ratas adultas con ciclos estrales regulares (ratas cíclicas) o con SOPQ sobre la concentración de progesterona, testosterona y estradiol en suero, y si tales efectos dependen de la información nerviosa aportada por el NOS. Ratas hembras adultas en cada día del ciclo estral, y ratas con SOPQ que presentaron un estro vaginal persistente, entre los 68 y 72 días de edad fueron sometidas o no a la sección unilateral del NOS seguida o no por la inyección de 20 µl de solución salina o VIP (10^{-6}M) en la bursa del ovario inervado o denervado. Los animales fueron sacrificados una hora después de la cirugía. La concentración de progesterona, testosterona y estradiol en suero fue cuantificada en todos los grupos.

En la rata cíclica, la inyección unilateral de VIP en la bursa ovárica resultó en una mayor concentración de testosterona y menor de estradiol en suero. Tales efectos dependen del ovario estimulado, del día del ciclo estral y de la integridad del NOS. En las ratas con SOPQ la

concentración de testosterona y estradiol fue mayor en comparación a su grupo control. La sección del NOS izquierdo resultó en una menor concentración de estradiol, mientras que en ratas con sección del NOS derecho se observó una menor concentración de progesterona y estradiol, y mayor de testosterona. Los efectos de la estimulación VIPérgica del ovario izquierdo o derecho sobre la concentración de progesterona, testosterona y estradiol también dependen de la integridad de la inervación del NOS y de la hormona evaluada.

Los presentes resultados nos permiten sugerir que en la rata adulta cíclica cada ovario tiene diferente sensibilidad a la estimulación VIPérgica que depende del estado endocrino del animal y de las señales nerviosas que ingresan a cada ovario a través del NOS. En la rata con SOPQ la regulación de la secreción aguda de hormonas esteroides por las señales VIPérgicas y noradrenérgicas (NAérgicas) del NOS es diferente de aquella en animales sin la patología. Con base en los presentes resultados se propone la existencia de una interacción funcional entre los neurotransmisores y neuropéptidos que arriban al ovario a través del NOS sobre la regulación de la secreción de hormonas esteroides que es diferente en la rata con SOPQ que en la rata cíclica.

Palabras clave: Síndrome del ovario poliquístico (SOPQ), nervio ovárico superior (NOS), péptido intestinal vasoactivo (VIP), ciclo estral, hormonas esteroides.

ABSTRACT

The ovarian functions are regulated by endocrine, immunological and neural sympathetic, sensorial and parasympathetic signals. The superior ovarian nerve (SON) is the main source of sympathetic innervations and provides the ovary with fibers containing neuropeptide Y (NPY), noradrenaline (NA) and vasoactive intestinal peptide (VIP).

Studies *in vitro* show in granulosa cells and in whole rat ovaries, the NA increases the progesterone, androstenedione and testosterone release while VIP enhances the progesterone, androgens and estradiol secretion. In rats with polycystic ovarian syndrome (PCOS) induced by injection of estradiol valerate (EV), the activity of SON fibers is higher than in control group, resulting in an increase of NA and VIP levels in the ovary. In culture of PCOS rat's ovaries, the NA adding increases the release of progesterone and androgens, while the addition of VIP enhances the secretion of androgens and estradiol. In PCOS rats the unilateral or bilateral sectioning of the SON normalizes testosterone and estradiol serum levels.

The aim of the present study was to analyze the acute effects of unilateral injection of VIP into the ovarian bursa of adult rats with regular estrous cycles (cyclic rats) or PCOS rats on progesterone, testosterone and estradiol serum levels and whether such effects depend of the neural information provided by the SON. Cyclic female rats on each day of estrous cycle, and PCOS rats presenting prolonged vaginal estrus, at 68-72 days old were submitted or not to the unilateral sectioning of the SON followed or not by the injection of 20 µl of saline solution or VIP (10^{-6}M) into the bursa of innervated or denervated ovary. The animals were sacrificed one hour after surgery. Progesterone, testosterone and estradiol serum levels were determined in all groups.

In the cyclic rat, the unilateral VIP injection into the ovarian bursa resulted in a higher testosterone and lower estradiol serum levels. Such effects depend on the stimulated ovary, the day of the estrous cycle and the integrity of the SON. In PCOS rats, sectioning the left SON resulted in lower estradiol levels, while sectioning the right one resulted in lower progesterone and estradiol levels, and higher testosterone levels than control. The effects of VIPergic

stimulation of the left or right ovary on progesterone, testosterone and estradiol levels also depended on the integrity of the SON innervation and the hormone evaluated.

The present results suggest that in the adult cyclic rat each ovary has different sensitivities to VIPergic stimulation which depends on the endocrine status of the animal and on the neural signal arriving to each ovary through the SON. In the PCOS rat the regulation of the acute secretion of steroid hormone by VIPergic and noradrenergic signals of the SON is different than in animals without the pathology. Based in present results we propose the existence of a functional interaction between neurotransmitters and neuropeptides arriving to the ovary through SON on steroid hormone secretion regulation that to be different in the PCOS rat than in cyclic rat.

Keywords: Polycystic ovarian syndrome (PCOS), superior ovarian nerve (SON), vasoactive intestinal peptide (VIP), estrous cycle, steroid hormone.

CAPÍTULO I

INTRODUCCIÓN GENERAL

El ovario realiza dos funciones: 1) la ovulación, que se define como la liberación de uno o varios ovocitos capaces de ser fecundados; y 2) la esteroidogénesis, que consiste en la secreción de hormonas esteroides (Domínguez y col., 1991; Domínguez y Cruz-Morales, 2011).

Las hormonas esteroides son lípidos que derivan de una molécula de colesterol. El ovario sintetiza tres tipos de hormonas esteroides: 1) Los *progestágenos*, tales como la pregnenolona, 17α-hidroxiprogesterona y progesterona; 2) Los *andrógenos*, como la androstenediona y testosterona; y 3) Los *estrógenos*, como la estrona y el 17β-estradiol (Gore-Langton y Armstrong, 1994; Norman y Litwack, 1997; Goldfien y Monroe, 2000; Strauss, 2009; Hu y col., 2010).

La esteroidogénesis ovárica es un proceso regulado por señales neuroendocrinas que se originan en el eje hipotálamo-hipófisis-ovario. El hipotálamo secreta la hormona liberadora de gonadotropinas (GnRH) que es vertida al sistema porta-hipotalámico-hipofisiario mediante el cual la GnRH ingresa a la adenohipófisis. El incremento en la frecuencia de los pulsos de liberación de GnRH estimula en los gonadotropos la secreción de la hormona luteinizante (LH), mientras que su disminución estimula la producción de la hormona folículo-estimulante (FSH) (Gore-Langton y Armstrong, 1994; Strauss, 2009; Hall, 2009; Dicken y col., 2010).

En el ovario, ambas gonadotropinas activan la vía de señalización del adenosín monofosfato cíclico/proteína cinasa A (AMPc/PKA) que resulta en una respuesta esteroidogénica aguda, crónica o en ambas. La respuesta aguda ocurre en cuestión de minutos como resultado de la rápida movilización de las reservas de colesterol celular hacia las mitocondrias; mientras que los efectos crónicos involucran la transcripción de genes para enzimas esteroidogénicas, por lo que ocurren en tiempos prolongados (Hu y col., 2010; Miller y Auchus, 2011). En células tecales y en células granuloso-luteínicas, la LH estimula la síntesis de progesterona, mientras que en células tecales-intersticiales incrementa la producción de andrógenos. La secreción de estradiol por las células de la granulosa es regulada por acción de la FSH. Los esteroides gonadales modulan la secreción de GnRH y de gonadotropinas por

retroalimentación positiva o negativa sobre el eje hipotálamo-hipófisis (Tajima y col., 2007; Jamnongjit y Hammes, 2006; Strauss, 2009; Hall, 2009; Kiener, 2010).

El ovario es inervado por fibras de naturaleza simpática, sensorial y parasimpática que participan en la modulación de sus funciones (Lawrence y Burden, 1980; Burden, 1985; Dissen y col., 2004; Domínguez y Cruz-Morales, 2011).

La porción simpática de la inervación ovárica se origina en los segmentos T11 a L4 de la médula espinal y hace sinapsis en el ganglio celiaco-mesentérico superior (GCMS) (Burden, 1978; Lawrence y Burden, 1980; Burden, 1985; Dissen y Ojeda, 1999), localizado en la pared ventral de la aorta abdominal (Anesetti y col., 2009). En el GCMS se originan dos paquetes nerviosos, el nervio del plexo ovárico (NPO), que viaja a lo largo de la arteria ovárica y el nervio ovárico superior (NOS), que se encuentra asociado al ligamento suspensorio (Lawrence y Burden, 1980; Dissen y Ojeda, 1999).

El NOS es de naturaleza simpática, transporta neuropéptido Y (NPY), noradrenalina (NA) y péptido intestinal vasoactivo (VIP) e inerva estructuras como el ovario, el oviducto y el útero (Baljet y Drukker, 1979; Lawrence y Burden, 1980; Bahr y Ben-Jonathan, 1981; Dees y col., 1986; Schultea y col., 1992). En el ovario, las fibras noradrenérgicas (NAérgicas) del NOS se encuentran asociadas a los vasos sanguíneos, a las células de la glándula intersticial y a la teca interna (Baljet y Drukker 1980; Burden, 1972; Lawrence y Burden, 1980; Hsueh y col., 1984; Erikson y col., 1985; Spicer, 1986). Las células de la granulosa y del cuerpo lúteo carecen de inervación (Hsueh y col., 1984; Erikson y col., 1985), sin embargo responden ante un estímulo catecolaminérgico debido a la presencia de receptores adrenérgicos (Adashi y Hsueh 1981; Aguado y col., 1982; Ratner y col., 1980).

La estimulación eléctrica del NOS incrementa la concentración ovárica de progesterona a través de la activación de los receptores β-adrenérgicos y la inhibe mediante la activación de los receptores α-adrenérgicos (Weiss y col., 1982). *In vitro*, la estimulación de células de la granulosa, tecales-intersticiales o del ovario completo de la rata con agonistas β-adrenérgicos resulta en una mayor secreción de progesterona, androstenediona y testosterona, pero no de

estradiol (Ratner y col., 1980; Adashi y Hsueh, 1981; Aguado y col., 1982; Dyer y Erickson, 1985; Hernandez y col., 1988; Selvaraj y col., 2000). Según Garraza y col., (2004), la incubación de ovarios de ratas en diestro 1 con NA disminuye la secreción de progesterona, mientras que la incrementa en ovarios obtenidos de ratas en diestro 2, lo que indica una respuesta diferente del cuerpo lúteo a la estimulación adrenérgica en cada día del diestro.

La sección bilateral del NOS realizada a ratas prepúberes resulta en una menor concentración de NA ovárica (Aguado y Ojeda, 1984b). En el día del proestro se observa una inmediata disminución de la secreción de progesterona y estradiol en respuesta a la sección bilateral del NOS (Aguado y Ojeda, 1984a). Los efectos agudos de la sección unilateral del NOS realizada en cada día del ciclo estral sobre la secreción de progesterona, testosterona y estradiol son asimétricos y varían en función del día del ciclo estral y de la hora del día en que se realice la cirugía (Flores y col., 2011; Ramírez, 2011). Las asimetrías han sido explicadas como resultado de la diferencia en la información nerviosa que ingresa a los ovarios a través del NOS. El grupo de Morán (2005) mostró que existe asimetría en la actividad de las conexiones nerviosas entre los ovarios y el GCMS, y que el número de neuronas activas de estas conexiones varía durante el ciclo estral de la rata.

El VIP en el ovario tiene dos orígenes, principalmente es de origen extrínseco, aportado en su mayoría por el NOS (Dees y col., 1986) y en menor proporción por el nervio vago (NV) (Said y Rosenberg, 1976; Dees y col., 1986). Gozes y Tsafriri, (1986) mostraron la presencia del ARNm de VIP en ovarios de rata, por lo que propusieron que el péptido se sintetiza de manera local. Es posible que el péptido sea sintetizado en las células de la granulosa ya que en folículos preovulatorios de vaca se observaron células de la granulosa inmunoreactivas al VIP (Hulshof y col., 1994). Al igual que las fibras NAérgicas, en el ovario de la rata las fibras VIPérgicas se encuentran asociadas a los vasos sanguíneos, a la glándula intersticial, a folículos en reposo y a la teca de folículos en desarrollo (Ahmed y col., 1986; Dees y col., 1986).

Existen dos tipos de receptores transmembranales a VIP, el VPAC-1 y el VPAC-2 (Ishihara y col., 1992; Lutz y col., 1993). Los efectos del VIP en las funciones del ovario son mediados por

la activación del AMPc/PKA (Davoren y Hsueh, 1985; George y Ojeda, 1987; Johnson y col., 1994; Gräs y col., 2000; Romero y col., 2002).

El VIP participa en la regulación de enzimas que intervienen en la síntesis de hormonas esteroides donde: 1) incrementa la expresión y fosforilación de la proteína reguladora de la esteroidogénesis aguda (StAR) (Kowalewski y col., 2010); 2) aumenta la síntesis del ARNm (Johnson y col., 1994) y de la proteína (Trzeciak y col., 1986) del complejo enzimático P450scc; 3) estimula la actividad de la enzima 3β-hidroxiesteroide deshidrogenasa (3β-HSD) y disminuye la actividad de la 20α-hidroxiesteroide deshidrogenasa (20α-HSD) (Davoren y Hsueh, 1985); 4) incrementa la síntesis del ARNm de la enzima 17α-hidroxilasa (Johnson y col., 1994); y 5) estimula la actividad de las aromatasas (George y Ojeda, 1987).

Diversos estudios *in vitro* e *in vivo* muestran que el VIP regula la síntesis de hormonas esteroides. En cultivo de células de la granulosa o de ovarios completos de ratas, el VIP incrementa la síntesis de progesterona y estradiol (Davoren y Hsueh, 1985; Ahmed y col., 1986; Parra y col., 2007), así como la secreción de andrógenos por ovarios completos de ratas cíclicas (Parra y col., 2007) y por la teca de folículos preovulatorios del ovario de gallina (Tilly y Johnson, 1989). En la coneja, la infusión intravenosa de VIP resulta en un rápido incremento en la concentración plasmática de progesterona, sin afectar la de testosterona o estradiol (Fredericks y col., 1983). Previamente mostramos que a las 24 horas de la microinyección unilateral de VIP en la bursa ovárica de ratas en cada día del ciclo estral, las concentraciones de progesterona, testosterona y estradiol en suero varían dependiendo del ovario tratado y del ambiente endocrino del animal (Rosas, 2011).

En cultivo de ovarios de ratas de 4 días de edad, el VIP disminuye la población de folículos primordiales e incrementa la de folículos en crecimiento. Los autores mostraron que el VIP también incrementa el ARNm del ligando kit (kit-L), que promueve la transición del folículo primordial a primario, por lo que propusieron que el mecanismo a través del cual el VIP estimula el inicio del crecimiento folicular es mediante la activación del Kit-L (Chen y col., 2013). Estudios *in vitro* muestran que la estimulación de ovarios de ratas neonatas de dos días de

edad con VIP o NA resulta en la síntesis de receptores de FSH biológicamente activos, lo que contribuye a la diferenciación de los folículos primordiales (Mayerhofer y col., 1997).

En folículos antrales de rata el VIP inhibe la picnosis nuclear y la descamación de las células de la granulosa, signos indicativos de atresia folicular. En células de la granulosa en cultivo, el agregado de VIP al medio disminuye el ADN de bajo peso molecular, indicador de apoptosis celular; este efecto antiapoptótico es mediado por el factor de crecimiento semejante a la insulina (IGF-I), que promueve la supervivencia folicular (Flaws y col., 1995). En células de la granulosa de folículos preovulatorios de ganso, el VIP regula a la alta la expresión del gen Bcl-2, un factor anti-apoptótico (Wang y col., 2012)

Por algunas evidencias se ha mostrado que el VIP participa en la regulación del proceso de ovulación. El VIP estimula la maduración meiótica del ovocito (Törnell y col., 1988) así como la expresión del activador tisular del plasminógeno (tPA) tanto en el complejo cúmulo-ovocito como en el estrato granuloso (Liu y col., 1987). El tPA es una proteasa que participa en la conversión de plasminógeno en su forma activa, la plasmina, enzima que degrada el colágeno de la pared folicular y facilita la ruptura del folículo y consecuentemente la liberación del ovocito (Yeh y Adashi, 2001). En ovarios de ratas prepúberes colocados en un sistema de perfusión *in vitro*, se ha mostrado que el VIP estimula de manera dosis dependiente la expulsión del ovocito, aunque el número de ovocitos presentes en el medio es menor que el inducido con LH (Schmidt y col., 1990).

Síndrome del Ovario Poliquístico en la Mujer

El Síndrome del Ovario Poliquístico (SOPQ) es el desorden endocrino y metabólico más común de la población femenina en edad reproductiva caracterizado por hiperandrogenismo, oligo-ovulación/anovulación, ovarios poliquísticos (OPQ), oligomenorrea/amenorrea, hirsutismo y una elevada proporción en la relación LH/FSH (Hoyt and Schmidt, 2004; Bremer, 2010; Bronstein y col., 2011; Goodarzi y col., 2011; Diamanti-Kandarakis y Dunaif, 2012; Baldani y col., 2015; Palomba y col., 2015).

El SOPQ se asocia con una alta incidencia de infertilidad, obesidad, resistencia a la insulina e hiperinsulinemia (Dunaif y col., 1989; Carmina y col., 1992; Legro y col., 1998; DeUgarte y col., 2005; Fica y col., 2008; Goodarzi y col., 2011; Diamanti-Kandarakis y Dunaif, 2012; Baldani y col., 2015). Las mujeres con SOPQ tienen mayor riesgo de presentar diabetes mellitus tipo 2 (Ehrmann y col., 1999; Legro y col., 1999) y desarrollar cáncer endometrial, ovárico o de mama (Gadducci y col., 2005; Navaratnarajah y col., 2008; Chittenden y col., 2009).

En 1935 Stein y Leventhal describieron que algunas mujeres presentaban OPQs asociados con amenorrea, hirsutismo, obesidad e infertilidad, lo cual fue conocido como "Síndrome Stein-Leventhal". Posteriormente este síndrome fue denominado como SOPQ (Merino y col., 2009; Diamanti-Kandarakis y Dunaif, 2012). Debido a la heterogeneidad del SOPQ, los signos bioquímicos y estructurales considerados para su diagnóstico han sido modificados, por lo que su incidencia depende de los criterios utilizados. Al día de hoy su diagnóstico se basa exclusivamente en criterios reproductivos como el hiperandrogenismo, oligo-ovulación/anovulación y presencia de OPQ (Merino y col., 2009; Goodarzi y col., 2011; Baldani y col., 2015; Palomba y col., 2015). Se estima que el SOPQ tiene una incidencia del 4 al 7% de acuerdo a lo establecido en 1990 por el Instituto Nacional de Salud Infantil y Desarrollo Humano (NICHD), (Zawadzki y col., 1992; Lim y col., 2013), del 15 al 18% según los criterios definidos en el año 2003 en un consenso realizado en Rotterdam (March y col., 2010; Mehrabian y col., 2011) y del 9% de acuerdo con lo establecido en el año 2006 por la Sociedad del SOPQ y Exceso de Andrógenos (AE-PCOS) (March y col., 2010).

Aún cuando se ha avanzado en el estudio del SOPQ, su etiología permanece desconocida, y se ha atribuido a factores como:

1. Anormalidades Neuroendocrinas

El SOPQ se caracteriza por el aumento en la frecuencia de los pulsos de la GnRH (Taylor y col., 1997), que se atribuye a la alteración en el mecanismo de retroalimentación negativo de las hormonas ováricas sobre el hipotálamo, donde posiblemente la progesterona sea el principal modulador de la frecuencia de los pulsos de la GnRH (Bremer, 2010).

El aumento en la frecuencia de los pulsos de GnRH (1 pulso/60 min, en comparación a 1 pulso/90 min que se presenta en mujeres sin la patología del SOPQ) estimula una mayor expresión del ARNm de la subunidad β de la LH (Taylor y col., 1997) y una mayor secreción de esta gonadotropina (Rebar y col., 1976; Waldstreicher y col., 1988; Taylor y col., 1997). Esto se acompaña de una deficiencia relativa en la concentración de FSH y el aumento en la proporción LH:FSH (Rebar y col., 1976; Waldstreicher y col., 1988; Taylor y col., 1997). Cerca del 70% de mujeres con el SOPQ muestran mayor frecuencia y amplitud de los pulsos de LH y de la relación LH:FSH (Goodarzi y col., 2011).

El incremento en la concentración de LH estimula la producción de andrógenos en las células tecales. La relativa deficiencia de FSH altera la aromatización de andrógenos a estrógenos en las células de la granulosa, así como el desarrollo y la maduración folicular. Esta alteración en la secreción de gonadotropinas ocasionan un estado de hiperandrogenismo y disfunción ovulatoria (Blank y col., 2006; Bremer, 2010).

2. Resistencia a la Insulina

La resistencia a la insulina se define como la disminución de la sensibilidad de las células a la insulina (Olivares y Arellano, 2008). Entre el 50 y el 80% de mujeres con el SOPQ tienen resistencia a la insulina (Dumitrescu y col., 2015) que se presenta independientemente del grado de adiposidad y de las concentraciones de andrógenos (Bremer, 2010). En las mujeres con el SOPQ, la resistencia a la insulina ocurre principalmente en tejidos periféricos como el músculo esquelético y los adipocitos (Taylor, 1998) y se manifiesta por una disminución en el transporte de glucosa al interior de la célula (Olivares y Arellano, 2008). En consecuencia aumenta la concentración de glucosa en sangre e incrementa la secreción de insulina, lo que resulta en hiperinsulinemia (Taylor, 1998).

En las pacientes con SOPQ se ha observado que el número y estructura de los receptores a la insulina así como la afinidad de unión de la hormona a su receptor es normal (Ciaraldi y col., 1992; Conway y col., 1994; Dunaif y col., 1995; Talbot y col., 1996). La resistencia a la

insulina se atribuye a una alteración en la cascada de señalización del receptor de la insulina (Taylor, 1998; Fica y col., 2008; Bremer, 2010). Una vez que la insulina se une a su receptor se produce la fosforilación de residuos tirosina, responsables de transmitir la señalización de la insulina para la regulación de eventos metabólicos dentro de la célula. La insulina promueve la translocación del transportador de glucosa GLUT4 desde compartimentos intracelulares hacia la membrana plasmática. La fosforilación en residuos de serina/treonina en respuesta a la insulina ocurre como un mecanismo que atenúa la señalización intracelular activada por la insulina (Olivares y Arellano, 2008). En las mujeres con el SOPQ disminuye la fosforilación de residuos tirosina e incrementa la fosforilación de residuos serina/treonina, lo que además de ocasionar resistencia a la acción metabólica de la insulina (Diamanti-Kandarakis y Dunaif, 2012; Pala y col., 2014), aumenta la actividad del complejo enzimático P450c17, enzima limitante en la biosíntesis de andrógenos (Zhang y col., 1995). Se ha sugerido que al menos en un subgrupo de pacientes con SOPQ, el mismo defecto en la fosforilación de la serina/treonina es el causante de la resistencia a la insulina y del hiperandrogenismo (Diamanti-Kandarakis y Dunaif, 2012).

La insulina ejerce varios efectos que contribuyen a la condición hiperandrogénica que caracteriza a las mujeres con el SOPQ (Fica y col., 2008; Bremer, 2010). La insulina en conjunto con la LH estimula en las células de la teca la sobre-expresión de la P450c17, enzima limitante en la biosíntesis de andrógenos. Además, disminuye la producción hepática de la globulina de unión a hormonas sexuales (SHBG) lo que resulta en el incremento de la concentración de testosterona libre (Rosenfield y col., 1990; Rosenfield, 1999; Franks y col., 2005; Diamanti-Kandarakis, 2008; Fica y col., 2008; Bremer, 2010; Goodarzi y col., 2011).

3. Hiperandrogenismo

En mujeres con SOPQ, la concentración de androstenediona y testosterona en suero es del 50 al 150% mayor que en las mujeres sin la patología (Franks, 2005). *In vitro*, las células tecales de mujeres con SOPQ sintetizan 20 veces más androstenediona, 10 veces más 17α-

hidroxiprogesterona y 5 veces más progesterona que la teca de mujeres sin la patología (Gilling-Smith y col., 1994).

La condición hiperandrogénica de las mujeres con SOPQ se debe al incremento prolongado de la LH y de la insulina en sangre (Fica y col., 2008; Bremer, 2010; Goodarzi y col., 2011).

El hiperandrogenismo juega un papel en la etiología del SOPQ, ya que el incremento en la concentración de andrógenos altera la sensibilidad del hipotálamo a la progesterona., lo que resulta en el incremento de la frecuencia de los pulsos de liberación de GnRH, que conlleva a una excesiva producción de LH y a una relativa deficiencia de FSH (Blank y col., 2006). En mujeres con SOPQ el bloqueo de los receptores de andrógenos con flutamida restaura la sensibilidad del hipotálamo a la progesterona y como resultado disminuye la frecuencia de los pulsos de secreción de GnRH (Eagleson y col, 2000).

En pre-adipocitos obtenidos de la grasa subcutánea abdominal de mujeres con o sin SOPQ, las catecolaminas estimulan la lipólisis, mientras que los andrógenos disminuyen los efectos de las catecolaminas (Anderson y col., 2002; Arner, 2005). Según Bremer (2010) estos efectos de los andrógenos pueden contribuir al desarrollo de obesidad en la parte superior del cuerpo, un factor de riesgo para el desarrollo de resistencia a la insulina.

La ausencia en la expresión de las aromatasas observada en las células de la granulosa de folículos antrales de mujeres con SOPQ (Takayama y col., 1996) podría favorecer el incremento de los andrógenos en plasma.

4. Factores Genéticos

La incidencia de OPQ en mujeres cuyas madres tienen SOPQ es mayor que en las hijas de mujeres sin la patología (Battaglia y col., 2002). Previo al comienzo de la pubertad, estas mujeres presentan un mayor volumen ovárico e hiperinsulinemia (Sir-Petermann y col., 2009).

En las mujeres con SOPQ se ha observado que existe una alteración en la expresión de genes que codifican para la proteína plasmática SHBG, la subunidad β de la LH, el receptor de andrógenos así como los genes de enzimas que participan en la esteroidogénesis incluyendo a la StAR, la P450scc, la P450c17α y las aromatasas (Michelmore y col., 1999; Kahsar-Miller y col., 2001; Wachs y col., 2008; Sir-Petermann y col., 2009; Bremer, 2010; Gur y col., 2015). La alteración en la expresión de algunos de estos genes y de sus proteínas puede predisponer al desarrollo de hiperandrogenismo, diabetes mellitus tipo 2 o ambas (Jakubowski, 2005).

5. Hiperactividad Nerviosa

El SOPQ está asociado con un incremento en la actividad de los nervios simpáticos del ovario. En 1969 se describió que los ovarios de mujeres con SOPQ presentan una mayor inmunofluorescencia a las catecolaminas, indicativo de una mayor densidad de las fibras nerviosas catecolaminérgicas (Semenova, 1969). El grupo de Heider (2001) mostró que en las mujeres con esta patología incrementa el número de fibras nerviosas inmunoreactivas a la tirosina hidroxilasa (TH), enzima limitante en la biosíntesis de catecolaminas.

En mujeres con el SOPQ, repetidos tratamientos con electroacupuntura de baja frecuencia inhiben la hiperactividad del sistema nervioso simpático (Stener-Victorin y col., 2009), inducen la ovulación en más de un tercio de las mujeres (Stener-Victorin y col., 2000a) y disminuyen la concentración de testosterona y la proporción de LH:FSH (Stener-Victorin y col., 2000a; Jedel y col., 2011)

Modelos experimentales para la inducción del SOPQ

El SOPQ es una patología multifactorial, razón que ha dificultado el desarrollo de un modelo experimental capaz de semejar las alteraciones reproductivas y metabólicas que caracterizan al SOPQ en la mujer. No obstante, algunos modelos experimentales han permitido el abordaje de aspectos específicos observados en el SOPQ humano.

SOPQ inducido por Exceso de Andrógenos

Diferentes andrógenos han sido utilizados para inducir el SOPQ, entre los que se encuentran la testosterona, la dehidroepiandrosterona (DHEA), el propionato de testosterona (PT) y la dihidrotestosterona (DHT) (Shi y Vine, 2012; Walters y col., 2012).

La inyección diaria de PT en ratas de 21 días de edad, durante 35 días resultó en el desarrollo de quistes foliculares, anovulación e incremento en la concentración de insulina, por lo que se sugirió que el exceso de testosterona puede conducir al desarrollo de hiperinsulinemia (Beloosesky y col., 2004). Resultados semejantes fueron observados en ratas de 21 días de edad con implante subcutáneo de DHT (un andrógeno no aromatizable) que después de 9 a 13 semanas presentaron OPQ, diestro vaginal constante, bajas concentraciones de progesterona, sin alteraciones en testosterona o estradiol. Además, el uso de DHT resultó en el incremento del peso corporal, acumulación de tejido adiposo subcutáneo e intra-abdominal, altas concentraciones de insulina y resistencia a la insulina (Manneras y col., 2007). Estas evidencias muestran que el modelo del animal androgenizado es adecuado no solo para abordar alteraciones ováricas, sino también las metabólicas que caracterizan al SOPQ.

Las alteraciones inducidas por los andrógenos en la morfología del ovario dependen de la edad en que sean administrados. La inyección de PT en ratas de 9 días de edad resulta en el desarrollo del OPQ y ausencia de cuerpos lúteos. Este cuadro no se presenta cuando el PT se administra a los 28 ó 56 días de vida. Con estas evidencias se sugirió que las alteraciones ocasionadas en el eje hipotálamo-hipófisis-ovario son el resultado de la exposición del cerebro a elevadas dosis de andrógenos durante su periodo de diferenciación (Tamura y col., 2005). Además, en ratas inyectadas con PT a los 2 días de vida incrementa la actividad proliferativa de la zona reticular de la adrenal (Da Silva y col., 2009) que se acompaña del aumento de su grosor (Da Silva y col., 2007), lo que puede contribuir al estado hiperandrogénico de los animales con SOPQ.

En ovejas, primates no humanos y en roedores, la exposición del feto a elevadas concentraciones de andrógenos resulta en el desarrollo de OPQ, anovulación,

hiperandrogenismo, hipersecreción de LH, hiperinsulinemia e incremento del peso corporal (Abbott y col., 2005, 2008, 2009; Padmanabhan y col., 2006; Demissie y col., 2008; Franks, 2009; Wu y col., 2010). Estos resultados apoyan la hipótesis de que la exposición a un exceso de andrógenos durante una ventana clave del desarrollo (incluyendo *in útero*) resulta en características del SOPQ en la vida adulta (Franks, 2009).

SOPQ inducido por Estrés Crónico

La respuesta del organismo a una condición de estrés es regulada por el sistema nervioso central y periférico. Los factores genéticos, ambientales y del desarrollo determinan la adaptación de un individuo. Las catecolaminas forman parte de los moduladores de la respuesta al estrés (Chrousos y Gold, 1992).

Ciertas evidencias experimentales muestran que el incremento en la actividad NAérgica del ovario por efecto de la exposición crónica a estrés puede ser otro factor etiológico del SOPQ:

1) La exposición crónica de ratas adultas a un doble estresor (frío y restricción de movimiento) durante tres semanas resulta en el incremento de la concentración de NA en el ganglio celiaco y una mayor liberación de la monoamina desde el ovario, lo que se correlaciona con el desarrollo de prequistes y el incremento de la secreción de andrógenos y estradiol por los ovarios de estos animales (Paredes y col., 1998).

2) En la rata adulta, el estrés crónico por frío resulta en alteraciones en la morfología del ovario, que parecen depender del tiempo de exposición del animal al estresor. Después de cuatro semanas de estrés (4°C/3hrs/día, durante 5días/semana), incrementa la concentración de NA en el ovario y disminuye la población folicular total, sin inducir la formación de quistes (Dorfman y col., 2003). Según Bernuci y col. (2008), cuando el estrés por frío se prolonga durante ocho semanas no se modifica la concentración ovárica de NA aunque disminuye el número de ovocitos liberados y se observan prequistes y quistes foliculares, por lo que se propone que la formación de quistes en respuesta al estrés con frío, resulta del incremento

inicial en la concentración ovárica de NA (que se produce a las cuatro semanas de estrés por frío), aún cuando posteriormente la concentración de la amina se normalice

3) En los ovarios de ratas de 4 días de edad, cuyas madres fueron sometidas a estrés crónico por frío durante toda la gestación, se observó un menor número de folículos en diferentes estadios del desarrollo y menor concentración del ARNm del factor de crecimiento neural (NGF) y de los receptores a FSH. A la pubertad, las concentraciones de estradiol y de NA así como el número de folículos preovulatorios fueron bajos, y se retrasó la edad de apertura vaginal. Los autores postulan que los cambios en la concentración de NA ovárica representan un menor desarrollo de los nervios simpáticos que podría ser el causante en el retraso de la capacidad reproductiva (Barra y col., 2014).

4) En ratas adultas sometidas a estrés por frío durante 3 semanas (4°C/3hrs/día, 5días/semana), la inyección de VE al término del periodo de estrés no indujo la formación de quistes en los ovarios, mientras que en los animales expuestos de manera simultánea a estrés por frío y a una inyección de VE se desarrolló OPQ. Los autores sugieren que la ausencia de OPQ se debe a la adaptación de los animales al estrés, lo que conlleva a un menor tono NAérgico central o periférico al ovario que permite la inhibición o atenuación de los síntomas del SOPQ (Zangeneh y col., 2011).

SOPQ inducido por Exceso de Estrógenos

Para inducir el SOPQ o el OPQ se utilizan ésteres de estradiol con acción prolongada, como el benzoato de estradiol, el valerato de estradiol (VE) y el dipropionato de estradiol. En la actualidad el VE es el estrógeno más utilizados para la inducción del SOPQ (Shi y Vine, 2012; Walters y col., 2012).

El VE es un estrógeno de larga actividad con una vida media de 15 días (Rosa-E-Silva y col., 2003). La inyección de una dosis de VE en ratas infantiles resulta en el adelanto del inicio de la pubertad, alteración del patrón del ciclo estral, anovulación, altas concentraciones de testosterona y estradiol (Rosas, 2006; Rosa-E-Silva y col., 2003; Morales-Ledesma y col., 2010),

así como bajas concentraciones de LH, FSH y una menor proporción de LH:FSH (Rosa-E-Silva y col., 2003).

En los ovarios aumenta la atresia folicular y se forman prequistes y quistes foliculares (Brawer y col., 1986; Rosa-E-Silva y col., 1993; Morales-Ledesma y col., 2010). De acuerdo con el grupo de Brawer (1986), las alteraciones en la dinámica del desarrollo folicular ocurren en dos etapas: en los primeros 28 días, después de la administración de VE, disminuye el número total de folículos sanos y de cuerpos lúteos, aumenta el número de folículos atrésicos y no se observan folículos de Graaf ni quistes foliculares. Entre los 28 y 56 días post-tratamiento con VE la población de folículos sanos y atrésicos no varía, y es hasta el día 60 cuando se observan los quistes foliculares.

Al igual que en el modelo del animal sometido a estrés por frío, en la rata tratada con VE se ha analizado la participación de la inervación simpática como un factor etiológico del SOPQ. La administración de una dosis de VE resulta en una mayor actividad de la tirosina hidroxilasa y una mayor capacidad de las terminales nerviosas para incorporar y liberar NA (Lara y col., 1993), evento que se acompaña del incremento en la concentración de la amina en el ovario (Barria y col., 1993; Lara y col., 1993; Rosa-E-Silva y col., 2003; Morales-Ledesma y col., 2010) y que precede la formación de quistes foliculares (Lara y col., 1993). El grupo de Luna (2012) mostró que en la rata sin la patología del SOPQ, la inyección subcutánea de isoproterenol, un agonista no selectivo de los receptores β-adrenérgicos, incrementa la secreción de andrógenos e induce la formación de prequístes y quistes foliculares, efectos que no se observan cuando se bloquean dichos receptores con propranolol. Los autores sugirieron que en la rata, la estimulación β-adrenérgica es un componente en la condición del OPQ (Luna y col., 2012).

En las ratas tratadas con VE incrementa el contenido del NGF (Lara y col., 2000; Stener-Victorin y col., 2000b; Parra y col., 2007) y de su receptor de baja afinidad, el p75 (Lara y col., 2000). Repetidos tratamientos con electro-acupuntura en los segmentos somáticos que corresponde a la inervación de los ovarios (T12-L2, S2-4), resultan en menor concentración de NGF en el ovario y disminuyen la hiperactividad de las fibras simpáticas, sin que reviertan la morfología del OPQ (Stener-Victorin y col., 2000b). En animales inyectados con VE, la inyección

de anticuerpos anti-NGF normaliza el ciclo estral, disminuye el número de prequistes y quistes foliculares y se observan cuerpos lúteos, indicativos del restablecimiento de la función ovulatoria (Lara y col., 2000).

En cultivo de ovarios de ratas tratadas con VE, el isoproterenol incrementa la secreción de progesterona y testosterona, pero no la de estradiol (Barria y col., 1993). La sección bilateral del NOS en ratas con SOPQ resulta en la disminución de NA en el ovario, se normaliza el ciclo estral, la producción de hormonas esteroides y la ovulación (Barria y col., 1993; Rosa-E-Silva y col., 2003). La sección unilateral del NOS restablece la ovulación únicamente en el ovario inervado, mientras que el denervado mantiene su condición quística aun cuando el contenido de NA disminuyó significativamente, por lo que se sugirió que en el SOPQ además de la hiperactividad NAérgica existen otros factores que contribuyen al desarrollo de la patología (Morales-Ledesma y col., 2010).

En los ovarios de animales con SOPQ incrementa cuatro veces el contenido de VIP respecto al grupo control en estro. *In vitro*, la estimulación los ovarios de estos animales con VIP resulta en el aumento de la secreción de andrógenos y estradiol (Parra y col., 2007). En la rata tratada con VE, la denervación farmacológica con guanetidina (GTD), un neurotóxico que destruye de manera selectiva las fibras NAérgicas periféricas, resulta en una mayor concentración de estradiol en suero, efecto que fue atribuido al VIP (Ruíz y col., 2008). Previamente mostramos que en ratas con SOPQ y sección unilateral del NOS, la inyección de VIP (10^{-6} M) en la bursa del ovario izquierdo denervado duplicó resultó, 24 horas después, en la duplicación de la concentración de testosterona en suero, efecto opuesto al observado cuando el péptido se inyectó en el ovario derecho denervado (Rosas, 2011), lo que nos sugiere que la participación del VIP en la regulación de la secreción de hormonas esteroides depende del ovario en estudio.

PLANTEAMIENTO DEL PROBLEMA

La información simpática que llega al ovario por el NOS regula la esteroidogénesis por medio del VIP y de la NA. El VIP estimula la síntesis de hormonas esteroides y sus efectos dependen del: 1) ambiente endocrino del animal durante el ciclo estral; y del 2) ovario en estudio, ya que cada gónada responde de manera diferente al estímulo VIPérgico en un mismo día del ciclo.

El SOPQ es una patología multifactorial considerada como la principal causa de infertilidad femenina. Por evidencias clínicas y experimentales se ha postulado que la hiperactividad crónica de las fibras simpáticas del ovario contribuye al desarrollo del SOPQ. En la rata con SOPQ inducido con VE incrementa el contenido de NA y VIP en los ovarios. En cultivo de ovarios de ratas con SOPQ, el agregado de VIP o isoproterenol aumenta la secreción de testosterona y estradiol. La eliminación de la información simpática por la sección unilateral o bilateral del NOS en ratas con SOPQ normaliza la concentración de hormonas esteroides, contrario a lo que sucede ante la destrucción selectiva de las neuronas NAérgicas periféricas con GTD que resultó en el aumento de la concentración de estradiol. En las ratas con SOPQ, los efectos de la estimulación VIPérgica del ovario denervado es asimétrica.

Con base en estas evidencias proponemos que la inervación NAérgica que recibe el ovario por medio del NOS modula de manera diferencial la respuesta esteroidogénica de cada gónada al estímulo VIPérgico. Para analizar esta posibilidad y si tales efectos dependen del ambiente endocrino del animal, en la rata adulta con o sin SOPQ se analizaron los efectos agudos de la inyección unilateral de VIP en la bursa del ovario inervado o denervado sobre la concentración de hormonas esteroides en suero.

Los resultados obtenidos ayudarán en la comprensión de la participación del VIP en la regulación aguda de la esteroidogénesis de animales con o sin el SOPQ y si en esta respuesta está comprometida la información NAérgica que llega por el NOS.

HIPÓTESIS

Dado que la secreción ovárica de hormonas esteroides es regulada de manera estimulante por las fibras NAérgicas y VIPérgicas del NOS, que la capacidad de secreción de hormonas esteroides por la gónada derecha e izquierda es diferente, y que en el animal con SOPQ el aumento del aporte de NA y VIP al ovario incrementa la secreción de andrógenos y estrógenos, entonces, la sobre-estimulación del sistema VIPérgico del ovario del animal cíclico o con SOPQ resultará en una mayor secreción de progesterona, testosterona y estradiol, evento que dependerá del ovario en estudio y de la integridad del NOS.

OBJETIVO GENERAL

Analizar la interacción de la información NAérgica y VIPérgica en la regulación de la secreción ovárica de hormonas esteroides en el animal cíclico y en el animal con SOPQ inducido con VE.

OBJETIVOS PARTICULARES

- En cada día del ciclo estral de la rata adulta y en el día del estro de la rata con SOPQ:
    - Evaluar los efectos agudos de la estimulación unilateral del ovario con VIP sobre la concentración de progesterona, testosterona y estradiol en suero.
    - Analizar en la rata con sección unilateral del NOS, los efectos agudos de la inyección de VIP en la bursa del ovario inervado o denervado, sobre la concentración de progesterona, testosterona y estradiol en suero.
- En el modelo del animal con SOPQ evaluar los efectos agudos de la sección unilateral del NOS, sobre la concentración de progesterona, testosterona y estradiol en suero.

CAPÍTULO II

Asymmetric Steroidogenic Response by the Ovaries to the Vasoactive Intestinal Peptide

Gabriela Rosas[1], María Isabel Ramírez[1], Rosa Linares[1], Angélica Trujillo[2], Roberto Domínguez[1] and Leticia Morales-Ledesma[1].

[1] Biology of Reproduction Research Unit, Physiology of Reproduction Laboratory, Facultad de Estudios Superiores Zaragoza, UNAM. AP 9-020, CP 15000, México, D.F, México. [2] Biology of Reproduction Research Unit, Physiology of Reproduction Laboratory, Escuela de Biología, Benemérita Universidad Autónoma de Puebla, Puebla, México.

Artículo Requisito. Publicado en la revista *Endocrine* (2015) 48: 968-977.

Endocrine (2015) 48:968–977
DOI 10.1007/s12020-014-0449-x

ORIGINAL ARTICLE

Asymmetric steroidogenic response by the ovaries to the vasoactive intestinal peptide

Gabriela Rosas · María Isabel Ramírez · Rosa Linares · Angélica Trujillo · Roberto Domínguez · Leticia Morales-Ledesma

Received: 21 August 2014 / Accepted: 7 October 2014 / Published online: 21 October 2014

Abstract In vitro the vasoactive intestinal peptide (VIP) stimulates progesterone, androgens, and estradiol secretion, and the effects are time-dependent. The present study analyzed the acute (1 h) and sub-acute (24 h) effects of unilateral injection of VIP into the ovarian bursa on each day of the estrous cycle on progesterone, testosterone, and estradiol serum levels. Cyclic 60-day-old virgin female rats on diestrus-1, diestrus-2, proestrus, or estrus were injected with saline or VIP 10^{-6} M into the left or right ovarian bursa. One hour after saline injection on each day of estrus cycle, progesterone levels were higher than in control animals. The acute effects of saline solution on testosterone and estradiol levels were asymmetric and varied during the estrous cycle. In comparison with saline groups, the effects of VIPergic stimulation on progesterone, testosterone, and estradiol serum levels depend on the time elapsed between treatment and autopsy and vary during the estrous cycle. An acute asymmetric response from the ovaries to the VIP was observed at diestrus-1, diestrus-2, and proestrus on progesterone and estradiol levels. The asymmetries on testosterone levels were observed at diestrus-1, diestrus-2, and estrus days. The present results suggest that in the cyclic rat, each ovary has different sensitivities to VIPergic stimulation which depends on the endocrine status of the animal.

Keywords VIP · Ovarian bursa · Asymmetry · Estrous cycle · Steroid hormone

G. Rosas · M. I. Ramírez · R. Linares · R. Domínguez · L. Morales-Ledesma (✉)
Biology of Reproduction Research Unit, Physiology of Reproduction Laboratory, Facultad de Estudios Superiores Zaragoza, UNAM, AP 9-020, CP 15000 Mexico, D.F., Mexico
e-mail: moralesledesma@yahoo.com.mx

A. Trujillo
Escuela de Biología, Benemérita Universidad Autónoma de Puebla, Edificio 112A Ciudad Universitaria, CP 72570, Puebla, Mexico

Introduction

In the ovary, the ovulation and steroid hormones secretion are regulated by hormonal and neural signals [1–6]. The ovary receives sympathetic innervation through two neural pathways: the superior ovarian nerve (SON), which travels along the suspensory ligament and the ovarian plexus nerve (OPN) accompanying the ovarian artery [7]. The SON is the main source of sympathetic innervations [8] and provides to the ovary with fibers containing catecholamines and vasoactive intestinal peptide (VIP) [9–11]. The vagus nerve also provides VIPergic innervation to the ovaries [12].

VIP is a 28-amino acid peptide [13]. The VIP-mRNA has been observed in the ovaries of rats [14], and the presence of VIP immuno-reactivity in the granulosa layer of the pre-ovulatory follicles suggests that these cells may be the site where VIP is synthesized by the ovary, although it cannot be excluded that VIP is internalized by granulosa cells [15]. VIP immune-reactive fibers are associated to blood vessels, the interstitial tissue, and are found around pre-antral and antral follicles [11, 16].

VIP acts on target specific cells by binding to class II G protein-coupled receptors family, VPAC-1 and VPAC-2, which activates the adenylate cyclase pathway and stimulates cAMP production [17]. Using RT-PCR and immunofluorescence analysis, it has been shown that the VPAC-1 receptors are present in the theca/interstitial cells, while

VPAC-2 receptors are present in both granulosa and theca/interstitial cells [18].

In the ovary, VIP stimulates the initiation of follicular growth [19, 20], the maturation of oocytes from isolated rat follicles [21], tissue-type plasminogen activator activity [22], and ovulation in PMSG-primed immature rat [23]. VIP also inhibits granulosa cells apoptosis [18, 24] and follicular atresia [24].

In female rabbits, injecting VIP intravenously increases progesterone plasma levels [25]. Adding VIP to cultured ovaries [16] or granulosa cells [26, 27] stimulates progesterone, androgens, and estradiol secretion; effects are dose- and time-dependent [27]. These effects have been explained by VIP's multiple roles in the regulation of steroidogenic enzymes, including its ability to increase the expression of acute steroidogenic regulatory protein (StAR) [28]; the synthesis of mRNA for cytochrome P450 cholesterol side-chain cleavage enzyme complex (P450scc) and for 17α-hidroxilase (17α-OH) [26]. VIP also enhances the activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) [27] and of aromatases [29].

These evidences indicate that VIP plays an important role in regulating female reproductive functions. The levels of VIP in the rat's ovary vary along the estrous cycle [30]. In in vitro ovaries, VIP's stimulating effects on androgen and estradiol secretion are higher in ovaries obtained from rats on proestrus [30].

Previously, using unilateral ovariectomized animals as a study model, we showed the ovaries' capacity to secrete progesterone, testosterone, and estradiol is asymmetric, and that this asymmetry varies along the estrous cycle [31–35].

To our knowledge, there are no studies analyzing the acute and sub-acute effects of injecting VIP into the ovarian bursa of rats on each day of the estrous cycle. The aim of the present study was to analyze the effects on progesterone, testosterone, and estradiol serum levels resulting from the unilateral VIPergic stimulation of the ovaries along the estrous cycle of the rat. For this purpose, and to mainly stimulate the target ovary, VIP was micro-injected into the ovarian bursa of the left or right ovary of adult rats on diestrus-1, diestrus-2, proestrus, or estrus. Effects were evaluated 1 or 24 h after treatment.

Materials and methods

Experiments were carried out in strict accordance with the Mexican Law of Animal Treatment and Protection Guidelines and the specifications in the Mexican Official Standard NOM-062-ZOO-1999. The Committee of the Facultad de Estudios Superiores Zaragoza approved the experimental protocols. All efforts were made to minimize the number of animals used and their suffering.

The study was performed using 60-day-old virgin female rats of the CIIZ-V strain from our own stock. The animals were kept under controlled lighting conditions 14/10 h light–dark cycle (lights on from 05:00 to 19:00 h), with free access to rat chow and tap water. Estrous cycles were monitored by cytological examination of daily vaginal smears performed at 09:00 h. Only rats showing at least two consecutive 4-day cycles were used in the experiment. All surgeries were performed between 10:30 and 11:30 h at the days of diestrus-1, diestrus-2, proestrus, or estrus. Ten animals were used in each experimental group.

Experimental groups

Rats were randomly assigned to one of the following groups:

Control group. Groups of cyclic-untouched rats were sacrificed between 10:30 and 11:30 h on the day of diestrus-1, diestrus-2, proestrus, or estrus.

VIP or saline solution treatment: The left or right ovary treatment consisted of injecting the saline solution (0.9 % w/v NaCl) or the VIP 10^{-6} M (Sigma Chem. Co., St. Luis, MO, USA) solution into the respective left or right ovarian bursa. The concentration of VIP was based on studies in rat performed by Ahmed et al. [16], and Davoren and Hsueh [27], where they showed that a dose of 10^{-6} M of VIP produces the maximum stimulation of progesterone, androgens, and estradiol secretion from granulosa cells [27] and the whole ovary [16].

Injection procedures were performed following previously described methodology [36–39]. In brief, animals on each day of the estrous cycle were anesthetized with ether, and subsequently a left or right dorso-lateral incision was performed two cm below the last rib. The incision affected skin, muscle, and peritoneum, allowing exposure of the left or right ovary. After exposing the left or right ovary, 20 µl of saline or VIP solution was injected into the left or right ovarian bursa with the aid of a 0.5 ml syringe with a 31G × 8 mm gauge needle. To prevent leakage of the saline or VIP solution and allow the injected solution to fully cover the ovary, the needle was kept in the bursa for 1 min after injection treatment. Subsequently, the ovary was carefully cleaned, dried, and returned to the abdominal cavity, and the wound was immediately sealed.

Autopsy procedures

Rats were sacrificed by decapitation either 1 h (acute effects) or 24-h after treatment (sub-acute effects). The blood of the trunk was collected, allowed to clot at room temperature for 30 min, and centrifuged at 3,000 rpm during 15 min. Serum was stored at −20 °C until progesterone, testosterone, and estradiol levels were measured using radioimmunoassay (RIA).

Table 1 Means ± SEM of progesterone serum levels (ng/ml) in control rats and animals injected with saline solution into the left or right ovarian bursa on diestrus-1, diestrus-2, proestrus, or estrus

Groups	Day of treatment			
	Diestrus-1	Diestrus-2	Proestrus	Estrus
Sacrificed 1 h after treatment				
Control	15.7 ± 0.7[c]	4.2 ± 0.7	5.5 ± 0.7	4.6 ± 0.3
Left saline	38.6 ± 0.5[a]	27.1 ± 2.0[a]	20.4 ± 1.3[a]	34.0 ± 1.5[a]
Right saline	38.7 ± 0.5[a]	18.5 ± 0.9[a,b]	32.4 ± 2.7[a,b]	34.4 ± 1.4[a]
Sacrificed 24 h after treatment				
Control	4.2 ± 0.7	5.5 ± 0.7	4.6 ± 0.3	15.7 ± 0.7
Left saline	19.5 ± 2.7[a]	4.9 ± 0.4	6.3 ± 0.6	15.9 ± 0.8
Right saline	25.7 ± 2.1[a]	3.8 ± 0.5	5.1 ± 0.4	25.1 ± 3.0[a,b]

Animals were sacrificed one or 24 h after treatment

[a] $p < 0.05$ vs. its respective control group

[b] $p < 0.05$ vs. its respective saline solution treatment on the left ovary

[c] $p < 0.05$ vs. control groups sacrificed on diestrus-2, proestrus, or estrus (MANOVA followed by Tukey's test)

Hormone assay

Serum progesterone, testosterone, and estradiol concentration were determined in duplicate in a single assay using RIA, with solid-phase kits purchased from Diagnostic Products (Los Angeles, CA, USA). Results are expressed in ng/ml (progesterone) and pg/ml (testosterone and estradiol). The intra- and inter-assay coefficients of variation and their standard deviation were 8.35 ± 3.9 and 9.45 ± 0.09 % for progesterone, 9.65 ± 4.5 and 10.2 ± 0.1 % for testosterone, and 8.12 ± 3.8 and 9.28 ± 0.09 % for estradiol, respectively.

Statistical analyses

Data on progesterone, testosterone, and estradiol serum levels were analyzed using multivariate analysis of variance (MANOVA), followed by Tukey's test. Differences in serum hormone levels between two groups were analyzed using the Student's *t* test. A probability value, lower than 0.05, was considered statistically significant.

Results

Acute and sub-acute effects of unilaterally injecting a saline solution into the ovarian bursa on progesterone, testosterone, and estradiol serum levels

Acute effects on progesterone: Compared to the control group on any day of estrus cycle, 1 h after animals were treated with the saline solution into the left or right ovary, progesterone serum levels were higher. On diestrus-2, saline solution treatment in the left ovary yielded the highest progesterone level increase. A similar effect was observed on proestrus, when saline solution treatment was performed in the right ovary (Table 1).

Sub-acute effects on progesterone: Compared to the control group, 24 h after saline solution treatment to either ovary on diestrus-1 resulted in higher progesterone levels. On estrus, 24 h after saline solution treatment of the right ovary also resulted in higher progesterone levels (Table 1).

Acute effects on testosterone: Compared to control animals on proestrus, testosterone levels were higher 1 h after saline solution treatment of the right ovary. Animals on estrus treated with the saline solution on the left ovary showed lower testosterone levels 1 h after treatment (Table 2).

Sub-acute effects on testosterone: 24 h after rats on diestrus-1 were treated with the saline solution on the right ovary; testosterone levels were higher than its corresponding control group. On diestrus-2, the same treatment resulted in lower testosterone levels. In turn, 24 h after rats on proestrus were treated with the saline solution on the left ovary; testosterone levels were lower than its corresponding control group. Rats treated on estrus with saline solution into the left or right ovary showed lower testosterone levels (Table 2).

Acute effects on estradiol: Compared to their respective control group, animals on diestrus-1 treated with saline solution in either ovary or those treated on diestrus-2 or proestrus in the left ovary showed higher estradiol levels 1 h after treatment. On estrus, 1 h after saline solution treatment to the left ovary resulted in lower estradiol levels (Table 3).

Sub-acute effects on estradiol: Compared to control animals, lower estradiol levels were observed; 24-h after rats on diestrus-1 were treated with the saline solution on either ovary. Lower estradiol levels were also observed in rats on diestrus-2 treated with the saline solution on the left ovary (Table 3).

Our results indicate that, compared to control groups, the saline solution treatment resulted in significant differences on progesterone, testosterone, and estradiol serum levels. Consequently, the effects of VIP treatment into the left or right ovarian bursa were compared with their respective saline treatment groups.

Acute and sub-acute effects on progesterone, testosterone, and estradiol serum levels resulting from unilateral VIPergic ovarian stimulation

VIP acute affects on progesterone: Compared to the corresponding saline solution group, animals on diestrus-1 with VIPergic stimulation to the right ovary showed lower

Table 2 Means ± SEM of testosterone serum level (pg/ml) in control rats and animals treated with saline solution into the left or right ovarian bursa on diestrus-1, diestrus-2, proestrus, or estrus

Groups	Day of treatment			
	Diestrus-1	Diestrus-2	Proestrus	Estrus
Sacrificed 1 h after treatment				
Control	50.8 ± 7.2	44.7 ± 2.7	112.9 ± 13.9[c]	30.9 ± 5.2
Left saline	41.4 ± 9.4	35.2 ± 5.4	153.0 ± 22.0	4.7 ± 0.3[a]
Right saline	47.9 ± 2.6	43.5 ± 7.6	206.0 ± 13.6[a]	21.6 ± 0.7[b]
Sacrificed 24 h after treatment				
Control	44.7 ± 2.7	112.9 ± 13.9	30.9 ± 5.2	50.8 ± 7.2
Left saline	55.6 ± 8.3	78.0 ± 10.9	16.3 ± 2.6[a]	27.8 ± 3.6[a]
Right saline	75.9 ± 2.8[a,b]	42.3 ± 3.7[a,b]	29.1 ± 4.2[b]	19.6 ± 4.6[a]

Animals were sacrificed one or 24 h after treatment

[a] $p < 0.05$ vs. its respective control group

[b] $p < 0.05$ vs. its respective saline solution treatment on the left ovary

[c] $p < 0.05$ vs. control groups sacrificed on diestrus-1, diestrus-2, or estrus (MANOVA followed by Tukey's test)

Table 3 Means ± SEM of estradiol serum level in control rats and animals treated with saline solution into the left or right ovarian bursa on diestrus-1, diestrus-2, proestrus, or estrus

Groups	Day of treatment			
	Diestrus-1	Diestrus-2	Proestrus	Estrus
Sacrificed 1 h after treatment				
Control	27.2 ± 3.5[c]	72.4 ± 12.8	103.9 ± 15.8	52.2 ± 4.4
Left saline	61.6 ± 11.8[a]	115.1 ± 12.5[a]	205.1 ± 21.0[a]	28.7 ± 1.0[a]
Right saline	54.6 ± 2.6[a]	53.7 ± 5.7[b]	120.3 ± 8.2[b]	40.8 ± 5.0
Sacrificed 24 h after treatment				
Control	72.4 ± 12.8	103.9 ± 15.8	52.2 ± 4.4	27.2 ± 3.5
Left saline	26.7 ± 3.0[a]	46.3 ± 1.9[a]	52.0 ± 6.1	22.6 ± 1.6
Right saline	26.5 ± 1.3[a]	77.1 ± 11.2[b]	42.3 ± 1.8	26.7 ± 2.0

Animals were sacrificed one or 24 h after treatment

[a] $p < 0.05$ vs. its respective control group

[b] $p < 0.05$ vs. its respective saline solution treatment on the left ovary

[c] $p < 0.05$ *vs.* control groups sacrificed on diestrus-2, proestrus, or estrus (MANOVA followed by Tukey's test)

progesterone levels while on diestrus-2 resulted in higher progesterone levels 1 h after treatment. In turn, animals on proestrus with VIPergic stimulation to the left ovary, sacrificed 1 h after treatment, showed higher progesterone serum levels (Fig. 1a).

VIP sub-acute effects on progesterone: 24 h after VIPergic stimulation of the right ovary of rats on diestrus-2 resulted in higher progesterone levels than its respective saline solution group (Fig. 1b).

VIP acute effects on testosterone: Compared to the corresponding saline solution group, VIPergic stimulation of the left ovary of rats on diestrus-1 or diestrus-2 resulted in higher testosterone level 1 h after treatment. VIPergic stimulation to either ovary of rats on estrus day also yielded higher testosterone levels (Fig. 2a).

VIP sub-acute effects on testosterone: Compared to the corresponding saline solution group, twenty-four hours after VIPergic stimulation to either ovary of rats on diestrus-1 or of left ovary of animals on estrus day yielded lower testosterone levels. VIPergic stimulation of the left ovary of rats on proestrus day resulted in higher testosterone levels (Fig. 2b).

VIP acute effects on estradiol: Compared to the corresponding saline solution group, 1 h after VIPergic stimulation of the right ovary of rats on diestrus-1 decreased estradiol serum levels. VIPergic stimulation to the left

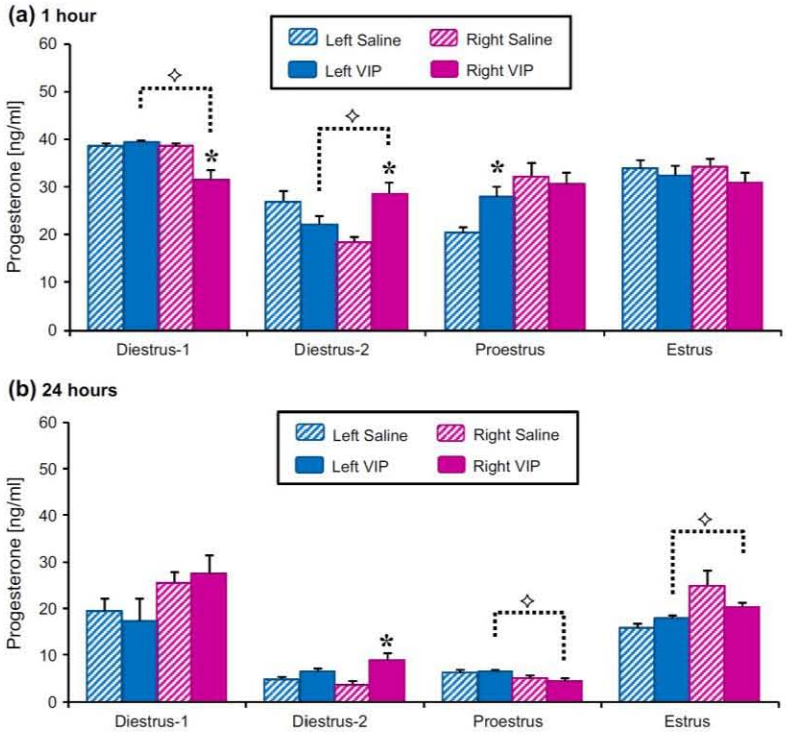


Fig. 1 Means ± SEM of progesterone serum level (ng/ml) in rats injected with saline or VIP into the left or right ovarian bursa on diestrus-1, diestrus-2, proestrus, or estrus. Animals were sacrificed 1 h (**a**) or 24 h (**b**) after treatment. *$p < 0.05$ vs. its respective saline solution group; ✧$p < 0.05$ *vs.* VIP solution group into the left ovary (Student's *t* test)

ovary of rats on diestrus-2 or proestrus also resulted in lower estradiol serum level (Fig. 3a).

VIP sub-acute effects on estradiol: Compared to the corresponding saline solution group, twenty-four hours after VIPergic stimulation of left ovary of rats on diestrus-2 or proestrus resulted in higher estradiol serum levels. In rats on diestrus-1 or proestrus, VIPergic stimulation of the right ovary also resulted in higher estradiol serum levels, while in rats on diestrus-2, VIPergic stimulation of the right ovary yielded lower estradiol serum levels (Fig. 3b).

Discussion

The results obtained in the present study show that in the adult rat, over-stimulating the VIPergic system of left or right ovary changes the secretion rates of steroid hormone (progesterone, testosterone and estradiol), and that these changes depend on the stimulated ovary, the day of the estrous cycle studied, and the time elapsed between the ovarian stimulation and autopsy.

Estradiol is the final product in the biosynthesis of steroid hormones by the ovary. In general, progesterone, testosterone, and estradiol levels are considered an activity index of key enzymes that participate in the synthesis of these three hormones: 3β-HSD for progesterone, P450c17α for testosterone, and aromatase for estradiol [3, 4].

In previous studies, we showed that the ovaries have an asymmetric capacity to release steroid hormones, and that this capacity varies along the estrous cycle [31, 33–35]. The ovaries' asymmetric response has been explained by the neural information received by each ovary [3]. Results obtained in the present study indicate that unilaterally injecting saline solution or VIP into the ovarian bursa modified the secretion rate of steroid hormones in different ways. These results suggest that the neural signals received by the ovary are translated differently by the left and right gonads, and that the information, or its translation, depends on the endocrine status of the animal.

Morphological evidences of a multisynaptic neural pathway between the brain and the adrenals, and between the brain and the ovaries have been reported [40]. Tóth et al. [41] described the existence of neurons in the central nervous system receiving direct neural communication from the adrenals and the ovaries. The unilateral perforation of the peritoneum to pre-pubertal [5, 6] or cyclic rats [31, 33] resulted in higher progesterone serum levels. In the present study, unilaterally injecting the saline solution into the bursa of either ovary on any day of the estrous cycle resulted in higher progesterone levels 1 h after treatment.

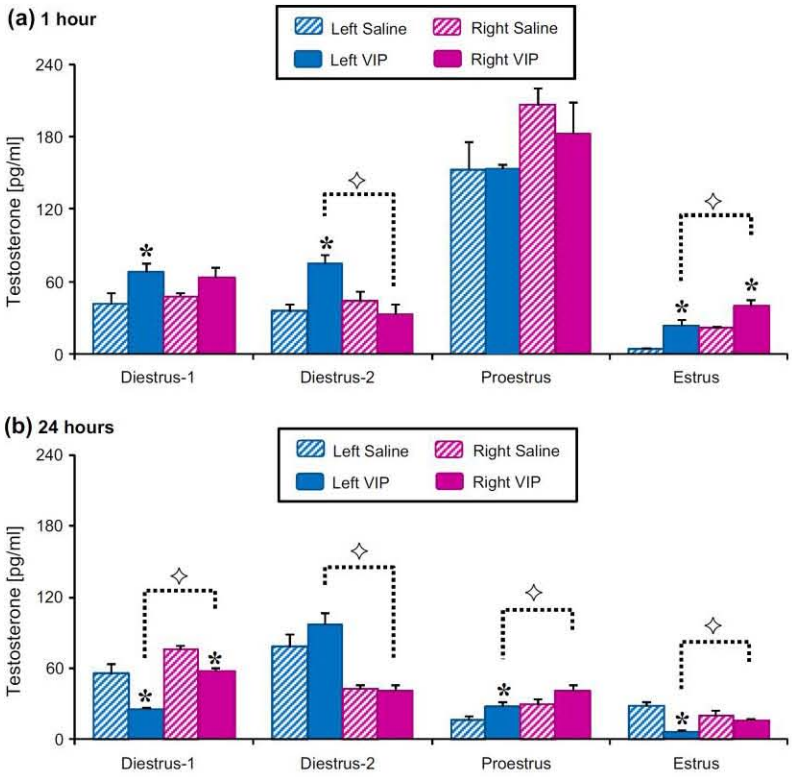


Fig. 2 Means ± SEM of testosterone serum level (pg/ml) in rats injected with saline or VIP into the left or right ovarian bursa on diestrus-1, diestrus-2, proestrus, or estrus. Animals were sacrificed 1 h (**a**) or 24 h (**b**) after treatment. $^{*}p < 0.05$ vs. its respective saline solution group; $\diamond p < 0.05$ *vs.* VIP solution group into the left ovary (Student's *t* test)

Since the adrenals seem to be the main source of progesterone secretion [42, 43], it is possible that the observed increase in progesterone levels to be mainly adrenal origin and result of a neural signal between ovaries and adrenals. Another possibility is that the distention of the ovarian bursa by saline solution activated a neural pathway connecting the ovaries and adrenals [41].This assumption must be further elucidated in detail.

VIP enhanced in vitro progesterone release in granulosa cell [27] or the whole ovary from pre-pubertal and puberal rats [16]. Such effects have been associated with increases in StAR mRNA levels and the protein phosphorylation [28]; higher P450scc mRNA and protein levels [26, 44]; and with greater rates of pregnenolone to progesterone conversion via 3β-HSD and lower metabolism of progesterone by 20α-hydroxysteroid dehydrogenase [27]. *In vitro*, adding VIP to hemi-ovaries from adult rats on diestrus-1 decreased the release of progesterone while increased it when the hemi-ovaries were obtained from rats on diestrus-2 [45]. In the present study, VIPergic stimulation yielded similar results, with an acute asymmetric response of progesterone secretion that varied along the estrus cycle. According to Hu et al. [46] and Domínguez et al. [3], the acute steroidogenic response is characterized by the phosphorylation (and hence the activation) of enzymes, a rapid mobilization of cholesterol esters and cholesterol delivery to the inner mitochondrial membrane, and its subsequent conversion to pregnenolone. Since VIP increases the expression and activity of key enzymes participating in the biosynthesis of progesterone [26–28, 44], it is possible that VIP inhibits or stimulates the activity of these enzymes depending on the neuroendocrine state of the animal.

During the estrous cycle of the rat, the ovaries are the main source of testosterone secretion, with only a moderate contribution by the adrenals [42, 47]. The acute effects on testosterone serum levels resulting from the unilateral perforation of the peritoneum depend on the side where the incision is made and the stage of the estrous cycle when surgery was performed [31, 34]. Similar results were obtained in the present study, where an asymmetric response on testosterone secretion was observed after unilateral saline solution treatment, which depends on the day of the cycle studied. Uchida et al. [48] showed that mechanical stimulation of abdominal skin result in the activation of spinal segmental reflex pathways that increase SON activity. According to Flores et al. [42], neural information arises from the dorsal-lateral peritoneum and

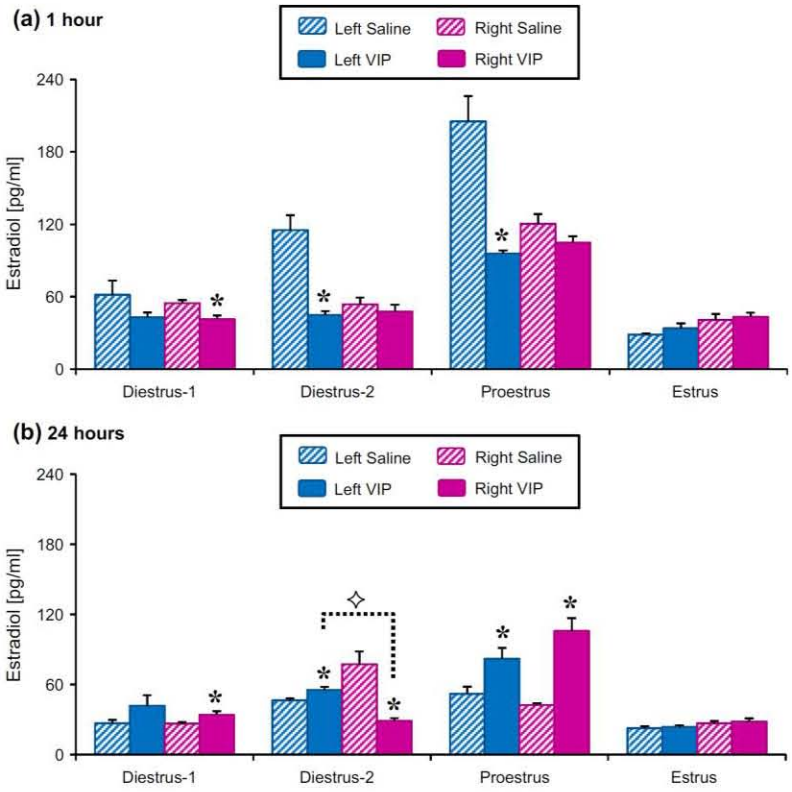


Fig. 3 Means ± SEM of estradiol serum level (pg/ml) in rats injected with saline or VIP into the left or right ovarian bursa on diestrus-1, diestrus-2, proestrus, or estrus. Animals were sacrificed 1 h (**a**) or 24 h (**b**) after treatment. $*p < 0.05$ vs. its respective saline solution group; $\diamond p < 0.05$ vs. VIP solution group into the left ovary (Student's t test)

from the ventral wall play different roles in the mechanism regulating testosterone secretion. Based on these evidences, it is possible that neural pathways arising from the abdominal skin or peritoneum that regulates enzymatic activity are activated during surgical procedures.

In in vitro rat ovaries, VIP has a stimulatory effect on androgen release [16, 30]. In cultured hen granulosa cells, the stimulating effects of VIP on androstenedione secretion are explained by the increase in 17α-OH mRNA levels [26]. VIP induces a maximum increase in androgen secretion in cultured rat ovaries obtained on early proestrus [30]. In the present study, comparing the effects of VIPergic stimulation with saline solution treatment indicates that VIP has an acute stimulatory effect on testosterone secretion and that the effect was asymmetric (between the left and right ovary) and depended on the day of the cycle studied.

On proestrus VIP's stimulatory effects on testosterone secretion are evident when comparing testosterone levels from VIPergic stimulated rats to those of intact animals, particularly in the right ovary. Compared to the mechanical/neural stimulation induced by the saline solution treatment on proestrus, the apparent lack of effects on testosterone serum levels resulting from VIPergic treatment suggests that the stimulation induced by the ovarian bursa distention reached the ovaries' maximum response capacity. This idea is supported by the fact that in rats anesthetized with ether, the increase in progesterone levels is similar to those in rats submitted to laparotomy [33]. Such results suggest that the hypothalamus–pituitary–adrenal axis' stress response capacity, manifested by increasing progesterone secretion, reaches its peak with the effects of ether anesthesia [33].

During the rat's estrous cycle, the intra-ovarian nerve growth factor (NGF) and VIP levels vary in an inverse relationship, suggesting that the local production of VIP is regulated by NGF [30]. According to Johnson et al. [26], in in vitro granulosa cells, VIP stimulates 17α-OH mRNA levels, and such effects are blocked with the addition of transforming growth factor α. Since the ovaries have an asymmetric capacity for testosterone release and this capacity varies along the estrous cycle [31, 34], we propose that the absence of acute effects on testosterone levels resulting from VIPergic stimulation to rats on diestrus-1 (right ovary), diestrus-2 (right ovary), or proestrus (both ovaries) could be explained by the action of local

neuro-trophic factors that modulate the effects of VIP in the ovary.

VIPergic innervation arrives to the ovaries mainly via the SON [11]. VIP enhances estradiol secretion in ovarian and granulosa cell cultures [16, 27, 30]. On estrus, electrical stimulation to the right SON during 5 min resulted in lower estradiol secretion rates in the ovarian venous blood [2], while bilateral section of the SON on proestrus day decreased estradiol secretion, with no apparent effects observed when SON surgery was performed on estrus day [49]. In the present study, the increase in estradiol serum levels that followed the unilateral injection of saline solution into the ovarian bursa on diestrus-2 or proestrus was inhibited with the VIPergic stimulation. Based on these results we propose that VIP has an asymmetric inhibitory effect on estradiol secretion at the ovarian level. It should be noted that the inhibitory effect of VIP on estradiol levels, observed 1 h after treatment, can also be related to hypothalamic control of ovarian VIP levels, as has been shown by Advis et al. [50] in pre-pubertal rats, the left, right, or bilateral lesions of the preoptic-anterior hypothalamic area resulted in higher VIP levels in the left ovary, suggesting the existence of a marked asymmetry in the hypothalamic control of ovarian VIP.

In the ovary, the follicle-stimulating hormone (FSH) stimulates proliferation of granulosa cells and the aromatization of testosterone to estrogens [51]. In fetal ovaries, VIP increases aromatase activity within 24 h and reached its maximal effect at 48 h [29]. The stimulating effects of VIP on estradiol production are time-dependent and of smaller magnitude than those induced by the FSH [27]. In the present study, unilateral overstimulation of the ovary with VIP on diestrus-1 or diestrus-2 resulted in asymmetric effects on estradiol secretion 24 h after treatment. The highest stimulatory effect of VIP on estradiol secretion was observed on proestrus day, similarly to the results reported by Parra et al. [30]. The ovarian follicle has a subpopulation of granulosa cells that are predominantly sensitive to VIP and another subpopulation that only responds to FSH stimulation [52]. Based on this information, we suggest that the different effects of VIP on each day of estrous cycle on estradiol serum levels observed in the present study are explained by the different sensitivities of the granulosa cell to the VIP in each stage of the estrus cycle.

According to Uchida et al. [48, 53, 54], the mechanical stimulation of the hindpaw or the abdominal wall decreased estradiol secretion rate by the ovary as a consequence of the reflex increase in SON activity. The decrease of the estradiol secretion rate from the ovary did not immediately result in changes in systemic blood [53]. In pre-pubertal rats, unilateral or bilateral laparotomy results in an acute (30 min) increase of progesterone and testosterone serum levels, while estradiol levels were lower. One hour after surgery, the serum levels of the hormones were higher than in untouched control animals [5, 6]. In present study, the sub-acute effects of unilateral saline injection into either ovarian bursa on steroid hormone levels could reflect the changes produced by neural signals arising from abdominal wall [48], ovaries, and the adrenals [40, 41, 55], which were activated during the surgery.

According to Hu et al. [46], long-term steroidogenesis stimulation induces the transcription of genes codifying for steroidogenic enzymes. Such increase in the synthetic capacity of the cells could explain the results observed 24 h after overstimulation of ovarian VIPergic system observed in the present study.

The asymmetries in hormone synthesis between the left and right ovaries observed in the present study could be explained by differences in the innervation received by each ovary. According to Tóth et al. [55], the supra-spinal innervation of the left ovary is more abundant than in the right ovary. Klein and Burden [56] showed a slight asymmetry in the number of neural fibers projecting into the ovary through the SON and OPN from the celiac-superior mesenteric ganglia, with a higher number of inputs into the right ovary than in the left. Additionally, Gerendai et al. [40] and Tóth et al. [41] showed that the supra-spinal innervations of the ovaries and adrenals have left side predominance.

Taken together, present results indicate that in the adult cyclic rat each ovary has a different sensitivity to VIPergic stimulation. It is possible that the ovarian asymmetric response to VIP is modulated, at least in part, by the innervation received by each ovary. The validity of this hypothesis awaits experimental analysis.

Acknowledgments This work was supported by grant UNAM-DGAPA-PAPIIT IN211813. We want to thank for the support given to in the realization of this study to the "Posgrado en Ciencias Biológicas, UNAM" and CONACyT. This work is a requirement for obtaining the degree of Doctor of Biological Sciences. We also thank Biol. R. Chavira having participated in performing the RIA's to measure the hormones levels. We also want to thank M Sc A. Domínguez-González for the revision of the manuscript in English.

Conflict of interest The authors declare that there is no conflict of interest.

References

1. M.L. Forneris, L.I. Aguado, Neonatal superior ovarian nerve transection disturbs the cyclic activity of the female rats. J. Steroid Biochem. Mol. Biol. **82**, 75–82 (2002)
2. F. Kagitani, S. Uchida, H. Hotta, Effects of electrical stimulation of the superior ovarian nerve and the ovarian plexus nerve on the ovarian estradiol secretion rate in rats. J Physiol Sci. **58**, 133–138 (2008)

3. R. Domínguez, A. Flores, S.E. Cruz-Morales, in *Hormonal and Neural Mechanisms Regulating Hormone Steroids Secretion*, ed. by A. Hassan. Steroids Basic Science, Chaper 1, 1st edn. (In Tech, Croacia, Rijeka, 2011), pp. 3–34. ISBN 978-953-307-866-3 (2011)
4. W.L. Miller, R.J. Auchus, The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr. Rev. **32**, 81–151 (2011)
5. L. Morales-Ledesma, D.A. Ramírez, E. Vieyra, A. Trujillo, R. Chavira, M. Cárdenas, R. Domínguez, Effects of acute unilateral ovariectomy to pre-pubertal rats on steroid hormones secretion and compensatory ovarian responses. Reprod. Biol. Endocrinol. **9**, 41 (2011)
6. L. Morales-Ledesma, E. Vieyra, D.A. Ramírez, A. Trujillo, R. Chavira, M. Cárdenas, R. Domínguez, Effects on steroid hormones secretion resulting from the acute stimulation of sectioning the superior ovarian nerve to pre-pubertal rats. Reprod. Biol. Endocrinol. **10**, 88 (2012)
7. I.E. Lawrence Jr, H.W. Burden, The origin of the extrinsic adrenergic innervation to the rat ovary. Anat. Rec. **196**, 51–59 (1980)
8. B. Baljet, J. Drukker, The extrinsic innervation of the abdominal organs in the female rat. Acta Anat. (Basel) **104**, 243–267 (1979)
9. J.M. Bahr, N. Ben-Jonathan, Preovulatory depletion of ovarian catecholamines in the rat. Endocrinology **108**, 1815–1820 (1981)
10. L.I. Aguado, S.R. Ojeda, Prepubertal ovarian function is finely regulated by direct adrenergic influences. Role of noradrenergic innervation. Endocrinology. **114**, 1845–1853 (1984)
11. W.L. Dees, C.E. Ahmed, S.R. Ojeda, Substance P- and vasoactive intestinal peptide-containing fibers reach the ovary by independent routes. Endocrinology **119**, 638–641 (1986)
12. S.I. Said, R.N. Rosenberg, Vasoactive intestinal peptide: abundant immunoreactivity in neural cell lines and normal nervous tissue. Science **192**, 907–908 (1976)
13. S.I. Said, V. Mutt, Polypeptide with broad biological activity: isolation from small Intestine. Science **169**, 1217–1218 (1970)
14. I. Gozes, A. Tsafriri, Detection of vasoactive intestinal peptide-encoding messenger ribonucleic acid in the rat ovaries. Endocrinology **119**, 2606–2610 (1986)
15. S.C.J. Hulshof, G. Dijkstra, E.M. Van Der Beek, M.M. Bevers, J.R. Figueiredo, J.F. Beckers, R. Van Den Hurk, Immunocytochemical localization of vasoactive intestinal peptide and neuropeptide Y in the bovine ovary. Biol. Reprod. **50**, 553–560 (1994)
16. C.E. Ahmed, W.L. Dees, S.R. Ojeda, The immature rat ovary is innervated by vasoactive intestinal peptide (VIP)-containing fibers and responds to VIP with steroid secretion. Endocrinology **118**, 1682–1689 (1986)
17. S. Onoue, S. Misaka, S. Yamada, Structure-activity relationship of vasoactive intestinal peptide (VIP): potent agonists and potential clinical applications. Naunyn-Schmiedeberg's Arch. Pharmacol. **377**, 579–590 (2008)
18. S. Vaccari, S. Latini, M. Barberi, A. Teti, M. Stefanini, R. Canipari, Characterization and expression of different pituitary adenylate cyclase-activating polypeptide/vasoactive intestinal polypeptide receptors in rat ovarian follicles. J. Endocrinol. **191**, 287–299 (2006)
19. A. Mayerhofer, G.A. Dissen, M.E. Costa, S.R. Ojeda, A role for neurotransmitters in early follicular development: induction of functional follicle-stimulating hormone receptors in newly formed follicles of the rat ovary. Endocrinology **138**, 3320–3329 (1997)
20. N. Chen, Y. Li, W. Wang, Y. Ma, D. Yang, Q. Zhang, Vasoactive intestinal peptide can promote the development of neonatal rat primordial follicles during in vitro culture. Biol. Reprod. **88**, 12 (2013)
21. J. Törnell, B. Carlsson, T. Hillensjö, Vasoactive intestinal peptide stimulates oocyte maturation, steroidogenesis, and cyclic adenosine 3′, 5′-monophosphate production in isolated preovulatory rat follicles. Biol. Reprod. **39**, 213–220 (1988)
22. Y.X. Liu, B.G. Kasson, K.D. Dahl, A.J.W. Hsueh, Vasoactive intestinal peptide stimulates plasminogen activator activity by cultured rat granulosa cells and cumulus-oocyte complexes. Peptides **8**, 29–33 (1987)
23. G. Schmidt, J. Jörgensen, P. Kannisto, F. Liedberg, B. Ottesen, Ch. Owman, Vasoactive intestinal polypeptide in the PMSG-primed immature rat ovary and its effect on ovulation in the isolated rat ovary perfused in vitro. J. Reprod. Fertil. **90**, 465–472 (1990)
24. J.A. Flaws, A. DeSanti, K.I. Tilly, R.O. Javid, K. Kugu, A.L. Johnson, A.N. Hirshfield, J.L. Tilly, Vasoactive intestinal peptide-mediated suppression of apoptosis in the ovary: potential mechanisms of action and evidence of a conserved antiatretogenic role through evolution. Endocrinology **136**, 4351–4359 (1995)
25. C.M. Fredericks, L.E. Lundquist, R.S. Mathur, S.H. Ashton, S.C. Landgrebe, Effects of vasoactive intestinal peptide upon ovarian steroids, ovum transport, and fertility in the rabbit. Biol. Reprod. **28**, 1052–1060 (1983)
26. A.L. Johnson, Z. Li, J.A. Gibney, S. Malamed, Vasoactive intestinal peptide-induced expression of cytochrome P450 cholesterol side-chain cleavage and 17α-hydroxylase enzyme activity in hen granulosa cells. Biol. Reprod. **51**, 327–333 (1994)
27. J.B. Davoren, A.J.W. Hsueh, Vasoactive intestinal peptide: a novel stimulator of steroidogenesis by cultured rat granulosa cells. Biol. Reprod. **33**, 37–52 (1985)
28. M.P. Kowalewski, M.T. Dyson, A. Boos, D.M. Stocco, Vasoactive intestinal peptide (VIP)-mediated expression and function of steroidogenic acute regulatory protein (StAR) in granulosa cells. Mol. Cell. Endocrinol. **328**, 93–103 (2010)
29. F.W. George, S.R. Ojeda, Vasoactive intestinal peptide enhances aromatase activity in the neonatal rat ovary before development of primary follicles or responsiveness to follicle-stimulating hormone. Proc. Natl. Acad. Sci. USA **84**, 5803–5807 (1987)
30. C. Parra, J.L. Fiedler, S.L. Luna, M. Greiner, V. Padmanabhan, H.E. Lara, Participation of vasoactive intestinal polypeptide in ovarian steroids production during the rat estrous cycle and in the development of estradiol valerate-induced polycystic ovary. Reproduction **133**, 147–154 (2007)
31. A.I. Barco, A. Flores, R. Chavira, P. Damián-Matsumara, R. Domínguez, M.E. Cruz, Asymmetric effects of acute hemiovariectomy on steroid hormone secretion by the in situ ovary. Endocrine **21**, 209–215 (2003)
32. M.E. Cruz, A. Flores, M.T. Palafox, G. Meléndez, J.O. Rodríguez, R. Chavira, R. Domínguez, The role of the muscarinic system in regulation estradiol secretion varies during the estrous cycle: the hemiovariectomized rat model. Reprod. Biol. Endocrinol. **4**, 43 (2006)
33. A. Flores, G. Meléndez, M.T. Palafox, J.O. Rodríguez, A.I. Barco, R. Chavira, R. Domínguez, M.E. Cruz, The participation of the cholinergic system in regulating progesterone secretion through the ovarian-adrenal crosstalk varies along the estrous cycle. Endocrine **28**, 145–151 (2005)
34. A. Flores, J.O. Rodríguez, M.T. Palafox, G. Meléndez, A.I. Barco, R. Chavira, M.E. Cruz, R. Domínguez, The acute asymmetric effects of hemiovariectomy on testosterone secretion vary along the estrous cycle. The participation of the cholinergic system. Reprod. Biol. Endocrinol. **4**, 11 (2006)
35. A. Flores, J. Velasco, A.I. Gallegos, F.D. Mendoza, P.M. Everardo, M.E. Cruz, R. Domínguez, Acute effects of unilateral sectioning the superior ovarian nerve of rats with unilateral ovariectomy on ovarian hormones (progesterone, testosterone and

estradiol) levels vary during the estrous cycle. Reprod. Biol. Endocrinol. **9**, 34 (2011)
36. R. Domínguez, M.E. Cruz, C. Morán, Differential effects of ovarian local anaesthesia during pro-oestrus on ovulation by the right or left ovary in normal and hemi-ovariectomized adult rats. J. Reprod. Fertil. **113**, 185–190 (1998)
37. C. Morán, A. Franco, J.L. Morán, A. Handal, L. Morales, R. Domínguez, Neural activity between ovaries and the prevertebral celiac-superior mesenteric ganglia varies during the estrous cycle of the rat. Endocrine **26**, 147–152 (2005)
38. C. Morán, F. Zarate, J.L. Morán, A. Handal, R. Domínguez, Lateralization of the connections of the ovary to the celiac ganglia in juvenile rats. Reprod. Biol. Endocrinol. **7**, 50 (2009)
39. M.J. Moran, M.E. Ayala, E. Gallegos, J. Romero, R. Chavira, P. Damián-Matsumura, R. Domínguez, Effects of systemic administration or intrabursal injection of serotonin on puberty, first ovulation and follicular development in rats. Reprod. Fertil. Dev. **25**, 1105–1114 (2013)
40. I. Gerendai, I.E. Tóth, Z. Boldogkoi, B. Halász, Recent findings on the organization of central nervous system structures involved in the innervation of endocrine glands and other organs; observations obtained by the transneuronal viral double-labeling technique. Endocrine **36**, 179–188 (2009)
41. I.E. Tóth, P. Banczerowski, Z. Boldogkoi, J.S. Tóth, A. Szabó, B. Halász, I. Gerendai, Cerebral neurons involved in the innervation of both the adrenal gland and the ovary: a double viral tracing study. Brain Res. Bull. **77**, 306–311 (2008)
42. A. Flores, A.I. Gallegos, J. Velasco, F.D. Mendoza, C. Montiel, P.M. Everardo, M.E. Cruz, R. Domínguez, The acute effects of bilateral ovariectomy or adrenalectomy on progesterone, testosterone and estradiol serum levels depend on the surgical approach and the day of the estrous cycle when they are performed. Reprod. Biol. Endocrinol. **6**, 48 (2008)
43. J. Kalász, E.P. Tóth, B. Bódi, M. Fagyas, A. Tóth, B.H. Pal, S.G. Vári, M. Balog, S. Blažetić, M. Heffer, Z. Papp, A. Borbély, Single acute stress-induced progesterone and ovariectomy alter cardiomyocyte contractile function in female rats. Croat. Med. J. **55**, 239–249 (2014)
44. W.H. Trzeciak, C.E. Ahmed, E.R. Simpson, S.R. Ojeda, Vasoactive intestinal peptide induces the synthesis of the cholesterol side-chain cleavage enzyme complex in cultured rat ovarian granulosa cells. Proc. Natl. Acad. Sci. USA **83**, 7490–7494 (1986)
45. M.H. Garraza, L.I. Aguado, M.A. De Bortoli, In vitro effect of neuropeptides on ovary or celiac ganglion affects the release of progesterone from ovaries in the rat. Med. Sci. Monit. **10**, 440–446 (2004)
46. J. Hu, Z. Zhang, W.J. Shen, S. Azhar, Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. Nutr. Metab. **7**, 47 (2010)
47. F. Stahl, F. Götz, G. Dörner, The influence of fetal adrenals on the androgen levels during brain differentiation in human subjects and rats. Exp. Clin. Endocrinol. **98**, 131–139 (1991)
48. S. Uchida, F. Kagitani, H. Hotta, T. Hanada, Y. Aikawa, Cutaneous mechanical stimulation regulates ovarian blood flow via activation of spinal and supraspinal reflex pathways in anesthetized rats. Jpn. J. Physiol. **55**, 265–277 (2005)
49. L.I. Aguado, S.R. Ojeda, Ovarian adrenergic nerves play a role in maintaining preovulatory steroid secretion. Endocrinology **114**, 1944–1946 (1984)
50. J.P. Advis, C.E. Ahmed, S.R. Ojeda, Direct hypothalamic control of vasoactive intestinal peptide (VIP) levels in the developing rat ovary. Brain Res. Bull. **22**, 605–610 (1989)
51. J.K. Findlay, K. Britt, J.B. Kerr, L. O'Donnell, M.E. Jones, A.E. Drummond, E.R. Simpson, The road to ovulation: the role of oestrogens. Reprod. Fertil. Dev. **13**, 543–547 (2001)
52. B.G. Kasson, R. Meidan, J.B. Davoren, A.J. Hsueh, Identification of subpopulations of rat granulosa cells: sedimentation properties and hormonal responsiveness. Endocrinology **117**, 1027–1034 (1985)
53. S. Uchida, F. Kagitani, H. Hotta, Reflex modulation of ovarian estradiol secretion by noxious mechanical stimulation of a hindpaw in anesthetized rats. Auton. Neurosci. **171**, 14–20 (2012)
54. S. Uchida, F. Kagitani, Autonomic nervous regulation of ovarian function by noxious somatic afferent stimulation. J. Physiol. Sci. (2014). doi:10.1007/s12576-014-0324-9
55. I.E. Tóth, O. Wiesel, Z. Boldogkoi, K. Bálint, Z. Tapaszti, I. Gerendai, Predominance of supraspinal innervation of the left ovary. Microsc. Res. Tech. **70**, 710–718 (2007)
56. C.M. Klein, H.W. Burden, Anatomical localization of afferent and postganglionic sympathetic neurons innervating the rat ovary. Neurosci. Lett. **85**, 217–222 (1988)

CAPÍTULO III

The Superior Ovarian Nerve modulates the Acute Steroidogenic Response of the Ovaries to the Vasoactive Intestinal Peptide during the Estrous Cycle of the Rat

Gabriela Rosas[1], Rosa Linares[1], Roberto Chavira[2], Roberto Domínguez[1] and Leticia Morales-Ledesma[1].

[1] Biology of Reproduction Research Unit, Physiology of Reproduction Laboratory, Facultad de Estudios Superiores Zaragoza, UNAM. AP 9-020, CP 15000, México, D.F, México. [2] Instituto Nacional de Ciencias Médicas y de la Nutrición "Salvador Zubirán", México.

Artículo en preparación para ser enviado a la revista *Endocrine*.

Abstract

In vitro the vasoactive intestinal peptide (VIP) increases the androgen and estradiol release by cyclic rat's ovaries. In cyclic rats, ovarian response to VIP injection in the ovarian bursa on steroid hormone release is asymmetric. In the present study we analyzed if the effects of VIPergic overstimulation depends on the superior ovarian nerve (SON) innervation. Cyclic female rats at 68-72 days old, on diestrus-1, diestrus-2, proestrus or estrus were submitted to unilateral sectioning of the SON and immediately after were microinjected with saline or VIP 10^{-6}M into the denervated or innervated ovary. Animals were sacrificed one hour after treatment. In comparison with saline groups, on diestrus-1 the VIPergic stimulation of the denervated left ovary resulted in lower progesterone, testosterone and estradiol levels; on diestrus-2 in higher estradiol and on proestrus in lower progesterone and testosterone and higher estradiol. VIP injection into the denervated right ovary resulted on diestrus-1 in higher testosterone levels, on diestrus-2 in higher estradiol and on estrus in lower testosterone and higher estradiol. The steroidogenic response of each ovary to VIPergic stimulation was different when the denervated ovary was the contralateral to the stimulation. The present results suggest that in the cyclic rat, the VIP effects on steroid hormone secretion are modulated by the neural signal arriving to the ovary through the SON that they are different for each ovary and depends on the endocrine status of the animal. The results also support the hypothesis of neural communication between the ovaries, possibly through the SON.

Keywords

Vasoactive Intestinal Peptide (VIP), Superior Ovarian Nerve (SON), Estrous Cycle, Progesterone, Testosterone, Estradiol.

Introduction

The sympathetic innervation of the mammalian ovary regulates steroidogenesis and ovulation [1-3]. The rat ovary is innervated by two sympathetic sources: the ovarian plexus nerve (OPN) that travels along the ovarian artery and the superior ovarian nerve (SON) which is associated with the suspensory ligament [4]. The neuronal bodies of the SON are located in the celiac–superior mesenteric ganglia (CSMG) and its nerve endings innervate predominately steroidogenic cells, particularly the thecal and interstitial cells and provides to the ovary neuropeptide Y, noradrenaline (NA) and vasoactive intestinal peptide (VIP) [4-10].

The neural control of ovarian steroidogenesis by the SON may be either excitatory through the stimulation of β-adrenergic receptors or inhibitory through of the α-adrenergic receptors [11]. The bilateral section of the SON causes a drop in ovarian NA content [12] that is companied by a lower progesterone and estradiol ovarian secretion [13].

In vitro studies show that the electrical stimulation of the ovary increases the release of NA, being higher during proestrus and estrus days [14]. The ovary of the rat contains a population of β-adrenergic receptors of the β2-subtype located in both granulosa and thecal cells of developing follicles and luteal cells. *In vitro*, the activation of these receptors with β-adrenergic agonists increases the progesterone and androgens secretion from whole ovary or ovarian cells [15-20].

In the rat's ovary the levels of VIP vary along the estrous cycle [21]. The theca/interstitial cells of the ovary express the VPAC-1 and VPAC-2 receptors, while VPAC-2 is expressed also in granulosa cells [22]. *In vitro*, VIP stimulates androgen and estradiol release by the ovaries [21]. The effects of adding NA, VIP, or NA+VIP to *in vitro* ovaries on progesterone production depend on the day of the estrous cycle of the rats [23]. The stimulation of α1-adrenergic receptors potentiates the progesterone release by rat granulosa cells stimulated with VIP [24]. In rats on each day of the estrous cycle, one or 24 hours after the microinjection of VIP into the left or right ovary was observed that the progesterone, testosterone and estradiol serum levels

are asymmetrical and depending on the day of the cycle [25]. These results suggest that ovarian response to VIP is modulated, at least in part, by the innervation received by each ovary [25]. The aim of present study was to analyze if the acute role of VIP on regulation of steroid hormone secretion depending on sympathetic innervation of the SON. For this purpose, cyclic rats with unilateral section of the SON were injected with VIP into the bursa of denervated or innervated ovary. The effects on progesterone, testosterone, and estradiol serum levels were evaluated one hour after treatment.

Materials and Methods

Experiments were carried out in strict accordance with the Mexican Law of Animal Treatment and Protection Guidelines and the specifications in the Mexican Official Standard NOM-062-ZOO-1999. The Committee of the Facultad de Estudios Superiores Zaragoza approved the experimental protocols. All efforts were made to minimize the number of animals used and their suffering.

The study was performed using 60-day-old virgin female rats of the CIIZ-V strain from our own stock. Rats were housed in acrylic cages under controlled conditions of temperature (22±2 °C) and light (lights on from 05:00 to 19:00 h), with free access to rat chow and tap water. Estrous cycles were monitored by cytological examination of daily vaginal smears performed at 09:00-10.00 h. Only rats showing at least two consecutive 4-day cycles were used in the experiment. Surgeries were performed under ether anesthesia between 10:30 and 11:30 h on diestrus-1, diestrus-2, proestrus, or estrus. All the animals were sacrificed by decapitation one hour after surgery. Ten rats were randomly assigned in each experimental group.

Experimental groups

The SON sectioning procedure was performed following previously described methodology [26]. Cyclic rats were anesthetized and a dorso-lateral incision on the left or right sides were performed 2 cm below the last rib, affecting skin, muscle and peritoneum. Then, the left or right ovary was exposed and the suspensory ligament (enclosing the SON) was sectioned at

approximately 1 cm from the ovary with the aid of fine forceps. Immediately after the unilateral section of the SON, the bursa of the denervated (ipsilateral ovary to the section of the SON) or innervated ovary (contralateral ovary to the section of the SON) was injected with 20 µl of saline solution (0.9 % w/v NaCl) or VIP 10^{-6} M solution (Sigma Chem. Co., St. Luis, MO, USA) with the aid of a 0.5 ml syringe with a 31G x 8mm gauge needle, following the methodology previously described [27], The concentration of VIP was the same one used previously [25]. To prevent leakage of the saline or VIP solution and allow the injected solution to fully cover the ovary, the needle was kept in the bursa for 1 min after injection. Subsequently, the ovary was carefully cleaned, dried, and returned to the abdominal cavity and the wound was sealed.

Autopsy procedures

At the autopsy, we collected the blood of the trunk in a test tube, allowed to clot at room temperature for 30 min, and centrifuged at 3,000 rpm during 15 minutes. Serum was stored at -20°C, until progesterone, testosterone and estradiol levels were measured using radioimmunoassay (RIA). To confirm the section of the SON, during autopsy it was verified that the ovary was free in the abdominal cavity.

Hormone assay

Serum levels of progesterone, testosterone, and estradiol were determined using RIA with solid-phase kits purchased from Diagnostic Products (Los Angeles, CA, USA) and the results expressed in ng/ml (progesterone) and pg/ml (testosterone and estradiol). The intra- and inter-assay coefficients of variation were 6.58% and 7.42% for progesterone, 7.85% and 8.76% for testosterone and 7.54% and 8.21% for estradiol, respectively.

Statistical analyses

Data on progesterone, testosterone, and estradiol serum levels were analyzed using the Student's t test. A probability value ≤ 0.05 was considered statistically significant.

Results

Effects of VIP injection into the *denervated* or innervated *ovary*

Progesterone

Denervated (ipsilateral) ovary. Compared to their corresponding saline group, VIPergic stimulation of the left ovary of rats on diestrus-1 or proestrus resulted in lower progesterone levels (Fig. 1).

Innervated (contralateral) ovary. In rats on diestrus-2 with section of the left SON, injecting VIP into right ovary resulted in higher progesterone levels than in saline group (Fig. 1).

Testosterone

Denervated (ipsilateral) ovary. On diestrus-1 or proestrus, the VIPergic stimulation of the left ovary resulted in lower testosterone levels than in saline group. When the injection of VIP was performed into the denervated right ovary on diestrus-1 testosterone levels were higher than saline-injected ones and lower when the injection was on the estrus day (Fig. 2).

Innervated (contralateral) ovary. In rats on diestrus-1 or 2, the VIP injection into the left ovary resulted in lower testosterone levels than in saline group (Fig. 2).

Estradiol

Denervated (ipsilateral) ovary. Estradiol levels were lower in rats with VIP injection into the left ovary on diestrus-1 and higher when the animals were treated on diestrus-2 or proestrus. VIP injection into the denervated right ovary of animals on diestrus-2 or estrus resulted in higher estradiol levels than saline injected ones (Fig. 3).

Innervated (contralateral) ovary. On diestrus-2 the injection of VIP on the left ovary resulted in higher estradiol levels than in saline group and lower when the treatment was in estrus. The injection of VIP into the right ovary of rats on diestrus-1, diestrus-2 or estrus resulted in lower estradiol levels and higher when the animals were treated on proestrus (Fig. 3).

In Figure 4 is shown a summary of the progesterone, testosterone and estradiol serum levels in cyclic rats resulting from the unilateral VIP injection into the left or right ovarian bursa or from the section of the left or right SON and injection of VIP into the denervated or innervated ovary.

Discussion

Present results show that the effects of VIP on secretion rates of progesterone, testosterone and estradiol by the ovaries depend on the integrity of the SON and vary during the estrous cycle of the rat.

VIP enhanced progesterone, androgens and estradiol release from granulosa cell and the ovary of pre-pubertal and pubertal rats [28, 29]. *In vitro*, the stimulation of ovaries from rats in diestrus-1 with VIP decreases the release of progesterone, while in ovaries obtained from rats on diestrus-2 stimulates it; on diestrus-1 the effects of VIP are amplified by NA but not in diestrus-2 [23]. The progesterone accumulation stimulated by VIP in cultured ovarian granulosa cells of rats was potentiated by a α_1-adrenergic agonist, but not by a β_2-adrenergic one [24]. Previously we showed that the unilateral VIPergic stimulation of the ovaries of cyclic rats results in an acute asymmetric response of steroid hormones secretion which varies along the estrus cycle [25]. Present results show that the response of the ovaries to the VIP depends on the integrity of the ovarian innervation of the SON and the day of estrous cycle, suggesting that neurotransmitters carried through the SON to the gonads modulate the effects of VIP on steroid hormones secretion in a different way for each hormone.

The unilateral section of the SON followed by the VIPergic stimulation of the contralateral ovary (innervated ovary) resulted in changes on progesterone, testosterone and estradiol secretion which depends on the ovary and the day of the cycle studied. These data suggest that neural signals arrive to the ovary from the contralateral ovary through the SON are indispensable for the ovary translate the VIPergic signal, and support the hypothesis of a neural communication between the ovaries as it has been proposed previously [30-32]. However we can not rule out that the exchange of information between the ovaries occurs through another nervous package, such as the OPN. Klein et al. [33] showed that electrical stimulation of the SON increased the frequency of action potentials in the OPN, suggesting that an ovarian neural reflex pathway exists and is mediated by the SON and the OPN.

The effects of sectioning the SON on steroid hormone levels are asymmetric and depend of the day of estrus cycle [34]. A similar effect was observed in the present study where the SON regulates asymmetrically the effects of VIP. These results could be explained by changes in the neural activity of the SON during the cycle. Morán et al. [32] showed that the number of active neurons between ovaries and the CSMG varies during the estrous cycle.

Taken together, present results indicate that in the adult cyclic rat the SON modulate of a stimulatory or inhibitory way the sensitivity of each ovary to VIPergic stimulation which depends on the day of the estrous cycle.

Acknowledgments

This work was supported by grant UNAM-DGAPA-PAPIIT IN211813. We want to thank for the support given to in the realization of this study to the "Posgrado en Ciencias Biológicas, UNAM" and CONACyT. The revision of the manuscript in English by M Sc Álvaro Domínguez-González is gratefully appreciated.

Declaration of interest

The authors declare that there is no conflict of interest.

References

1. Aguado, L.I.: Role of the central and peripheral nervous system in the ovarian function. Microsc. Res. Tech. 59, 462-473 (2002)
2. Lara, H.E., Dorfman, M., Venegas, M., Luza, S.M., Luna, S.L., Mayerhofer, A., Guimaraes, M.A., Rosa E Silva, A.A., Ramírez, V.D.: Changes in sympathetic nerve activity of the mammalian ovary during a normal estrous cycle and in polycystic ovary syndrome: Studies on norepinephrine release. Microsc. Res. Tech. 59, 495-502 (2002)
3. Domínguez, R., Cruz-Morales, S.E.: The ovarian innervation participates in the regulation of ovarian functions. Endocrinol. Metabol. Syndrome. (2011) doi:10.4172/2161-1017.S4-001
4. Lawrence, I.E. Jr., Burden, H.W.: The origin of the extrinsic adrenergic innervation to the rat ovary. Anat. Rec. 196, 51-59 (1980)

5. Baljet, B., Drukker, J.: The extrinsic innervation of the abdominal organs in the female rat. Acta Anat. (Basel). 104, 243-267 (1979)

6. Bahr, J.M., Ben-Jonathan, N.: Preovulatory depletion of ovarian catecholamines in the rat. Endocrinology. 108, 1815-1820 (1981)

7. Burden, H.W.: The adrenergic innervation of mammalian ovaries. In: Ben-Jonathan, N., Bahr, J.M., Weiner, R.I. (eds.) Catecholamines as hormone regulators, pp. 261-278. Raven Press, New York (1985)

8. Dees, W.L., Ahmed, C.E., Ojeda, S.R.: Substance P- and Vasoactive Intestinal Peptide-containing fibers reach the ovary by independent routes. Endocrinology. 119, 638-641 (1986)

9. Klein, C.M., Burden, H.W.: Anatomical localization of afferent and postganglionic sympathetic neurons innervating the rat ovary. Neurosci. Lett. 85, 217-222 (1988)

10. Schultea, T.D., Dees, W.L., Ojeda, S.R.: Postnatal development of sympathetic and sensory innervation of the rhesus monkey ovary. Biol. Reprod. 47, 760-767 (1992)

11. Weiss, G.K., Dail, W.G., Ratner, A.: Evidence for direct neural control of ovarian steroidogenesis in rats. J. Reprod. Fertil. 65, 507-511 (1982)

12. Aguado, L.I., Ojeda, S.R.: Prepubertal ovarian function is finely regulated by direct adrenergic influences. Role of noradrenergic innervation. Endocrinology. 114, 1845-1853 (1984)

13. Aguado, L.I., Ojeda, S.R.: Ovarian adrenergic nerves play a role in maintaining preovulatory steroid secretion. Endocrinology. 114, 1944-1946 (1984)

14. Ferruz, J., Barria, A., Galleguillos, X., Lara, H.E.: Release of norepinephrine from the rat ovary: local modulation of gonadotropins. Biol. Reprod. 45, 592-597 (1991)

15. Ratner, A., Weiss, G.K., Sanborn, C.R.: Stimulation by β_2-adrenergic receptors of the production of cyclic AMP and progesterone in rat ovarian tissue. J. Endocrinol. 87, 123-129 (1980)

16. Adashi, E.Y., Hsueh, A.J.: Stimulation of β_2-adrenergic responsiveness by follicle stimulating hormone in rat granulosa cells in vitro and in vivo. Endocrinology. 108, 2170-2178 (1981)

17. Aguado, L.I., Petrovic, S.L., Ojeda, S.R.: Ovarian β-adrenergic receptors during the onset of puberty: characterization, distribution and coupling to steroidogenic responses. Endocrinology. 110, 1124-1132 (1982)

18. Dyer, C.A., Erickson, G.F.: Norepinephrine amplifies human chorionic gonadotropin-stimulated androgen biosynthesis by ovarian theca-interstitial cells. Endocrinology. 116, 1645-1652 (1985)

19. Hernandez, E.R., Jimenez, J.L., Payne, D.W., Adashi, E.Y.: Adrenergic regulation of ovarian androgen biosynthesis is mediated via β_2-drenergic theca-interstitial cell recognition sites. Endocrinology. 122, 1592-1602 (1988)

20. Selvaraj, N., Dantes, A., Amsterdam, A.: Establishment and characterization of steroidogenic granulosa cells expressing β_2-adrenergic receptor: regulation of adrenodoxin and steroidogenic acute regulatory protein by adrenergic agents. Mol. Cell. Endocrinol. 168, 53-63 (2000)

21. Parra, C., Fiedler, J.L., Luna, S.L., Greiner, M., Padmanabhan, V., Lara, H.E.: Participation of vasoactive intestinal polypeptide in ovarian steroids production during the rat estrous cycle and in the development of estradiol valerate-induced polycystic ovary. Reproduction. 133, 147-154 (2007)

22. Vaccari, S., Latini, S., Barberi, M., Teti, A., Stefanini, M., Canipari, R.: Characterization and expression of different pituitary adenylate cyclase-activating polypeptide/vasoactive intestinal polypeptide receptors in rat ovarian follicles. J. Endocrinol. 191, 287-299 (2006)

23. Garraza, M.H., Aguado, L.I., De Bortoli, M.A.: *In vitro* effect of neuropeptides on ovary or celiac ganglion affects the release of progesterone from ovaries in the rat. Med. Sci. Monit. 10, 440-446 (2004)

24. Wasilewska-Dziubińska, E., Borowiec, M., Chmielowska, M., Woliñska-Witort, E., Baranowska, B.: Alfa 1 adrenergic potentiation of progesterone accumulation stimulated by vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) in cultured rat granulosa cells. Neuro. Endocrinol. Lett. 23, 141-148 (2002)

25. Rosas, G., Ramírez, M.I., Linares, R., Trujillo, A., Domínguez, R., Morales-Ledesma, L.: Asymmetric steroidogenic response by the ovaries to the vasoactive intestinal peptide. Endocrine. 48, 968-977 (2015)

26. Morales-Ledesma, L., Linares, R., Rosas, G., Morán, C., Chavira, R., Cárdenas, M., Domínguez, R.: Unilateral sectioning of the superior ovarian nerve of rats with polycystic ovarian syndrome restores ovulation in the innervated ovary. Reprod. Biol. Endocrinol. 8, 99 (2010)

27. Domínguez, R., Cruz, M.E., Morán, C.: Differential effects of ovarian local anaesthesia during pro-oestrus on ovulation by the right or left ovary in normal and hemi-ovariectomized adult rats. J. Reprod. Fertil. 113, 185-190 (1998)

28. Davoren, J.B., Hsueh, A.J.W.: Vasoactive intestinal peptide: a novel stimulator of steroidogenesis by cultured rat granulosa cells. Biol. Reprod. 33, 37-52 (1985)

29. Ahmed, C.E., Dees, W.L., Ojeda, S.R.: The immature rat ovary is innervated by vasoactive intestinal peptide (VIP)-containing fibers and responds to VIP with steroid secretion. Endocrinology. 118, 1682-1689 (1986)

30. Morales, L., Chávez, R., Domínguez, R.: Participation of the superior ovarian nerve in the regulation of ovulation in the prepubertal rat: differential effects of unilateral and bilateral section of the nerve. Med. Sci. Res. 21, 15-17 (1993)

31. Chávez, R., Morales, L., González, Ma.E., Domínguez, R.: Ovarian norepinephrine content in prepubertal rats with superior ovarian nerve section: temporal studies. Med. Sci. Res. 22, 789-790 (1994)

32. Morán, C., Franco, A., Morán, J.L., Handal, A., Morales, L., Domínguez, R.: Neural activity between ovaries and the prevertebral celiac-superior mesenteric ganglia varies during the estrous cycle of the rat. Endocrine. 26, 147-152 (2005)

33. Klein, C.M., Ray, R.H., Burden, H.W.: Direct electrical stimulation of the superior ovarian nerve in rats causes an increase in neuronal activity in the ipsilateral ovarian plexus nerve. Brain Res. 479, 194-200 (1989)

34. Flores, A., Velasco, J., Gallegos, A.I., Mendoza, F.D., Everardo, P.M., Cruz, M.E., Domínguez, R.: Acute effects of unilateral sectioning the superior ovarian nerve of rats with unilateral ovariectomy on ovarian hormones (progesterone, testosterone and estradiol) levels vary during the estrous cycle. Reprod. Biol. Endocrinol. 9, 34 (2011)

Fig. 1 Mean ± SEM of **progesterone** (ng/ml) serum level in cyclic rats with section of the left (L-SON) or right (R-SON) superior ovarian nerve and injected with saline or VIP into the denervated (ipsilateral) or innervated (contralateral) ovary. Animals were sacrificed 1 h after treatment. * $p<0.05$ vs. its respective saline group (Student's t test)

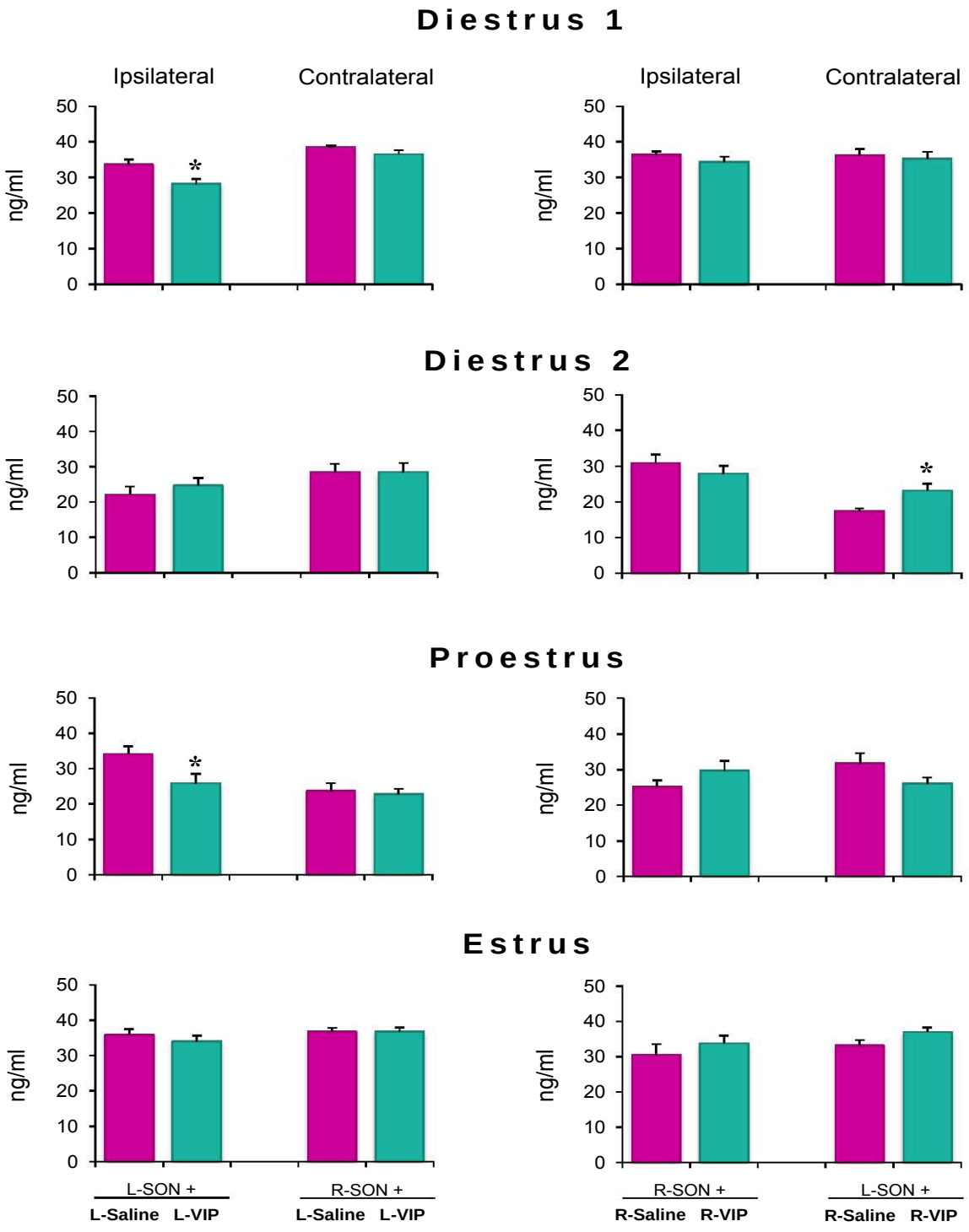


Fig. 2 Mean ± SEM of **testosterone** (pg/ml) serum level in cyclic rats with section of the left (L-SON) or right (R-SON) superior ovarian nerve and injected with saline or VIP into the denervated (ipsilateral) or innervated (contralateral) ovary. Animals were sacrificed 1 h after treatment. * $p<0.05$ vs. its respective saline group (Student's t test)

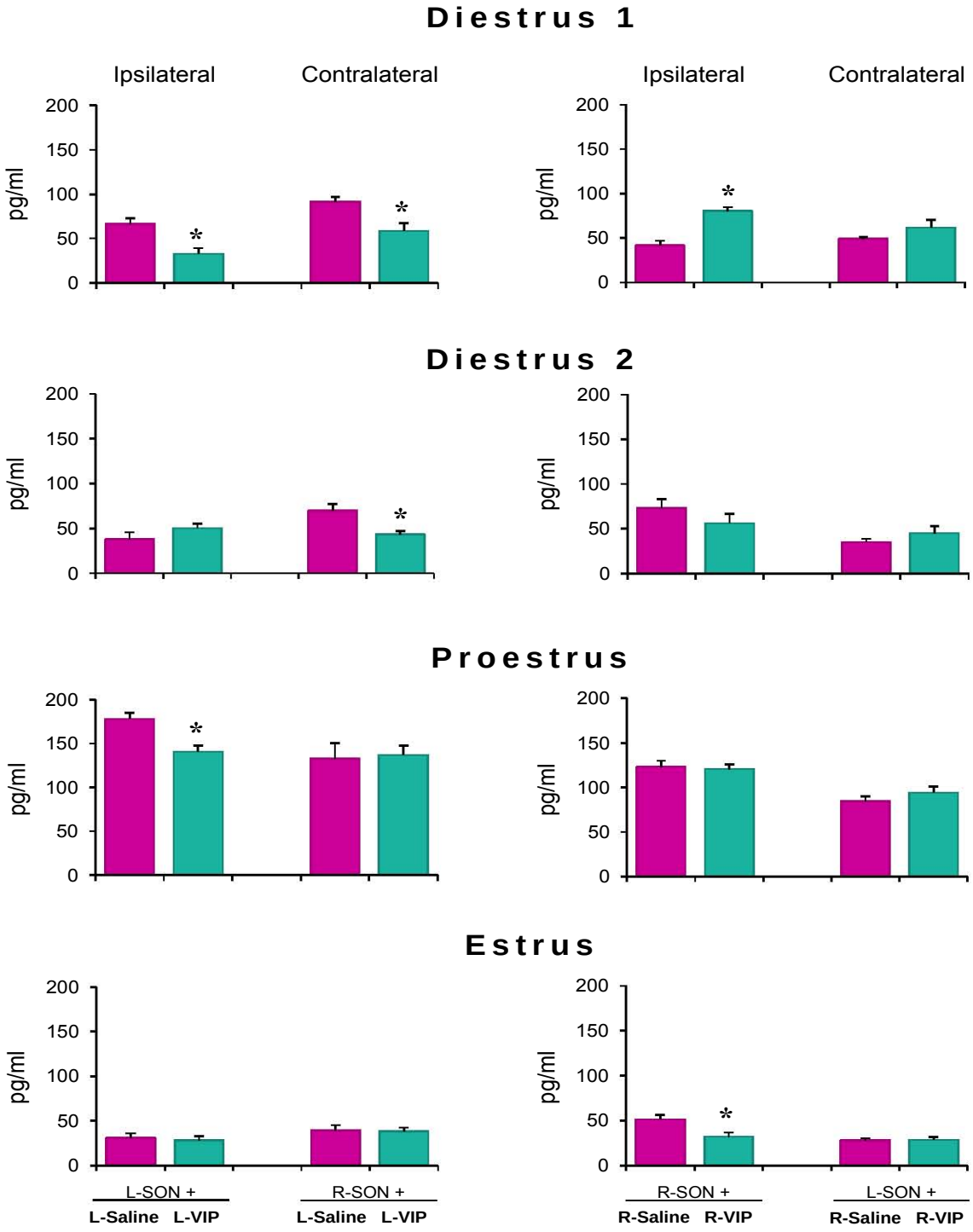


Fig. 3 Mean ± SEM of **estradiol** (pg/ml) serum level in cyclic rats with section of the left (L-SON) or right (R-SON) superior ovarian nerve and injected with saline or VIP into the denervated (ipsilateral) or innervated (contralateral) ovary. Animals were sacrificed 1 h after treatment. * $p<0.05$ vs. its respective saline group (Student's t test)

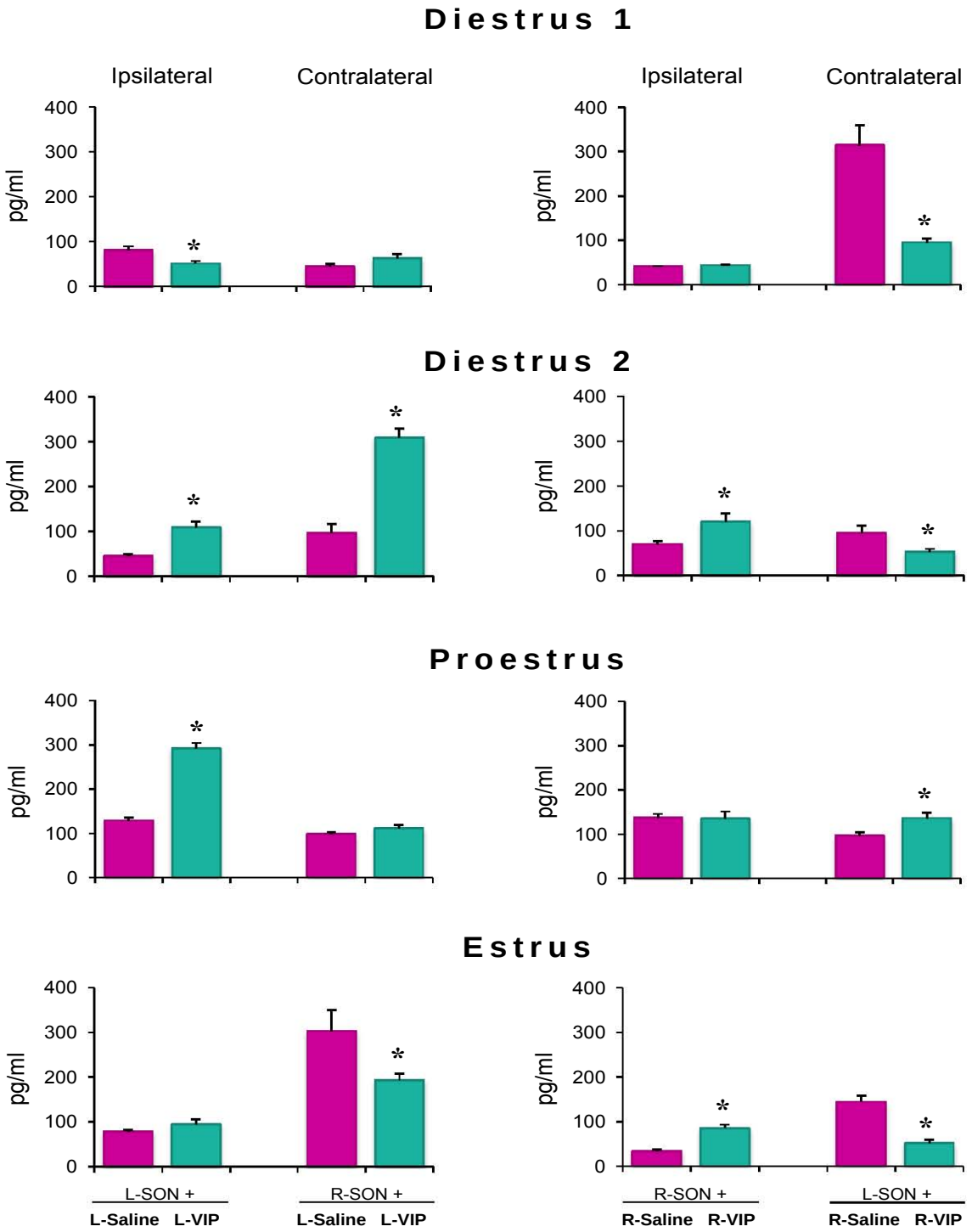


Fig. 4 Summary of injection of VIP into the denervated or innervated ovary of cyclic rats. Progesterone, testosterone and estradiol serum levels in cyclic rats resulting from the unilateral VIP injection into the left (L-VIP) or right (R-VIP) ovarian bursa (based on data published by Rosas et al. [2015]) or from the section of the left (L-SON) or right (R-SON) superior ovarian nerve and injected with VIP into the denervated (ipsilateral) or innervated (contralateral) ovary.

PROGESTERONE						
GROUPS	**L-VIP**	L-SON + **L-VIP**	R-SON + **L-VIP**	**R-VIP**	R-SON + **R-VIP**	L-SON + **R-VIP**
Diestrus-1	=	↓	=	↓	=	=
Diestrus-2	=	=	=	↑	=	↑
Proestrus	↑	↓	=	=	=	=
Estrus	=	=	=	=	=	=
TESTOSTERONE						
Diestrus-1	↑	↓	↓	=	↑	=
Diestrus-2	↑	=	↓	=	=	=
Proestrus	=	↓	=	=	=	=
Estrus	↑	=	=	↑	↓	=
ESTRADIOL						
Diestrus-1	=	↓	=	↓	=	↓
Diestrus-2	↓	↑	↑	=	↑	↓
Proestrus	↓	↑	=	=	=	↑
Estrus	=	=	↓	=	↑	↓

↑ Higher levels *vs.* saline group ↓ Lower levels *vs.* saline group

= No differences *vs.* saline group.

CAPÍTULO IV

In rats with Polycystic Ovary Syndrome the Vasoactive Intestinal Peptide role on the regulation of Steroid Hormone levels depends on the integrity of the Superior Ovarian Nerve

Gabriela Rosas[1], Lizzbeth De León-Gordillo[1], Rosa Linares[1], Roberto Chavira[2], Roberto Domínguez[1] and Leticia Morales-Ledesma[1].

[1] Biology of Reproduction Research Unit, Physiology of Reproduction Laboratory, Facultad de Estudios Superiores Zaragoza, UNAM. AP 9-020, CP 15000, México, D.F, México.
[2] Instituto Nacional de Ciencias Médicas y de la Nutrición "Salvador Zubirán", México.

Artículo enviado para su publicación a la revista *Endocrine*.

Endocrine

In rats with Polycystic Ovary Syndrome the Vasoactive Intestinal Peptide role on the regulation of steroid hormone levels depends on the integrity of the Superior Ovarian Nerve

--Manuscript Draft--

Manuscript Number:	
Full Title:	In rats with Polycystic Ovary Syndrome the Vasoactive Intestinal Peptide role on the regulation of steroid hormone levels depends on the integrity of the Superior Ovarian Nerve
Article Type:	Original Article
Corresponding Author:	Leticia Morales-Ledesma, PhD Facultad de Estudios Superiores Zaragoza, UNAM, Mexico, D.F MEXICO
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Facultad de Estudios Superiores Zaragoza, UNAM,
Corresponding Author's Secondary Institution:	
First Author:	Gabriela Rosas, PhD
First Author Secondary Information:	
Order of Authors:	Gabriela Rosas, PhD
	Lizzbeth De León-Gordillo, Biologist
	Rosa Linares, PhD
	Roberto Chavira, Biologist
	Roberto Domínguez, PhD
	Leticia Morales-Ledesma, PhD
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	Rats with polycystic ovarian syndrome induced by estradiol valerate injection (PCOS-EV) have higher noradrenaline (NA) and vasoactive intestinal peptide (VIP) ovarian levels. NA and VIP enhance steroid hormone release by cultured PCOS-EV rat's ovaries. The unilateral or bilateral sectioning of the superior ovarian nerve (SON) in PCOS-EV rats normalized testosterone and estradiol levels. In the present study we analyzed the acute effects on steroid hormone serum levels resulting from ovarian VIPergic overstimulation in PCOS-EV rats with or without unilateral sectioning of the SON. PCOS-EV rats at 68-72 days old, on vaginal estrus, were treated with 1) unilateral sectioning of the SON, 2) VIP (10-6M) injected into the left or right ovarian bursa, or 3) unilateral sectioning of the SON plus VIP injection into the denervated ovary. Animals were sacrificed one hour after surgery. Sectioning the left SON in PCOS-EV rats resulted in lower estradiol levels; while sectioning the right SON lowered progesterone and estradiol and increased testosterone levels. Injecting VIP into the left or right ovarian bursa lowered progesterone and estradiol, while in the right ovarian bursa increased testosterone levels. In rats with unilateral section of the SON, VIPergic stimulation of the left denervated ovary resulted in lower testosterone levels; while in the right denervated ovary lowered testosterone and estradiol levels. These results suggest that in PCOS-EV rats the neuroendocrine mechanisms regulating the acute secretion of ovarian steroid hormones depends on the VIPergic and noradrenergic signals arriving through the SON, and that these neural signals are different for each ovary.
Suggested Reviewers:	Hernán Lara, PhD

Abstract

Rats with polycystic ovarian syndrome induced by estradiol valerate injection (PCOS-EV) have higher noradrenaline (NA) and vasoactive intestinal peptide (VIP) ovarian levels. NA and VIP enhance steroid hormone release by cultured PCOS-EV rat's ovaries. The unilateral or bilateral sectioning of the superior ovarian nerve (SON) in PCOS-EV rats normalized testosterone and estradiol levels. In the present study we analyzed the acute effects on steroid hormone serum levels resulting from ovarian VIPergic overstimulation in PCOS-EV rats with or without unilateral sectioning of the SON. Control or PCOS-EV rats at 68-72 days old, on vaginal estrus, were treated with 1) unilateral sectioning of the SON, 2) VIP (10^{-6}M) injected into the left or right ovarian bursa, or 3) unilateral sectioning of the SON plus VIP injection into the denervated ovary. Animals were sacrificed one hour after surgery. In comparison with control group, sectioning the left SON in PCOS-EV rats resulted in lower estradiol levels; while sectioning the right SON lowered progesterone and estradiol and increased testosterone levels. Injecting VIP into the left or right ovarian bursa lowered progesterone and estradiol, while in the right ovarian bursa increased testosterone levels. In rats with unilateral section of the SON, VIPergic stimulation of the left denervated ovary resulted in lower testosterone levels; while in the right denervated ovary lowered testosterone and estradiol levels. These results suggest that in PCOS-EV rats the neuroendocrine mechanisms regulating the acute secretion of ovarian steroid hormones depends on the VIPergic and noradrenergic signals arriving through the SON, and that these neural signals are different for each ovary.

Keywords

Polycystic Ovarian Syndrome, Vasoactive Intestinal Peptide, Superior Ovarian Nerve, Progesterone, Testosterone, Estradiol.

Introduction

The polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, with an incidence of approximately 6–10% [1]. The etiology of PCOS is not completely understood. According to Matalliotakis et al. [2], three main hypotheses have been proposed on PCOS etiology: 1) A primary neuroendocrine defect leads to an exaggerated LH pulse frequency and amplitude; 2) An insulin action alteration leads to hyperinsulinemia; and 3) A defect of sex steroid synthesis or metabolism.

Based on clinical and experimental evidences, the hyper-activation of the ovary's sympathetic innervation has been proposed as a factor involved in PCOS etiology. Semenova [3] reported an increased fluorescence to catecholamines in ovaries from PCOS patients, while Heider et al. [4] demonstrated an increase in the number of tyrosine hydroxylase immunoreactive nerve fibers in the ovaries of women with the syndrome.

Pre-pubertal or adult rats injected with a single dose of estradiol valerate (EV), a long-acting estrogen, develop similar symptoms to human PCOS, such as acyclicity, anovulation, formation of ovarian cysts and higher androgen and estradiol levels [5-9]. In the ovaries of PCOS induced (PCOS-EV) animals, an increase in the activity of sympathetic nerves arriving to the ovary and in the ovarian noradrenaline (NA) content has been reported [10]. In rats, the *in vivo* stimulation of the β-adrenergic receptor by subcutaneous injection of isoproterenol resulted in higher androgens secretion and the presence of pre-cystic and cystic ovarian. Blocking β-adrenergic receptors prevented such effects [11].

The superior ovarian nerve (SON) is the main source of sympathetic innervation to the ovary and carries fibers containing NA and vasoactive intestinal peptide (VIP) [12-16]. In pre-pubertal and adult PCOS-EV rats the bilateral sectioning of the SON resulted in lower NA ovarian content, normalization of the estrous cycle, restoration of ovulation, and lower androgens and estradiol production [6, 8]. These results have been interpreted to suggest that a derangement of the sympathetic inputs to the ovary contributes to the development and maintenance of

PCOS [6, 8, 10]. Unilateral sectioning of the SON of PCOS-EV rats normalized testosterone and estradiol serum levels and restored ovulation in the innervated ovary; suggesting that aside from an increase in ovarian noradrenergic tone, other neural influences arriving to the ovaries via the SON participate in the PCOS pathogenesis [9].

According to Parra et al. [17] in PCOS-EV rats the ovarian VIP levels were higher than in control animals on estrus. In *in vitro* ovaries obtained from PCOS-EV rats, the release of androgen and estradiol increased after stimulation with VIP, suggesting that in PCOS-EV rats the VIP participates in the maintenance of increased ovarian steroids secretory activity [17].

In adult rabbits on estrus, immediately after the continuous infusion of 150 pmol of VIP during 60 min increased progesterone but not testosterone or estradiol plasma levels in comparison to those animals with an infusion of vehicle [18]. In *in vitro* mouse granulosa cells the release of progesterone increased after stimulation with VIP during six hours [19]. In addition, the androgen and estradiol release increased after two hours of stimulate with VIP the *in vitro* ovaries from rats in all cycle stages [17]. Previously we have shown that in rats on each day of the estrous cycle, one or 24 hours after microinjecting VIP into the left or right ovarian bursa was observed an acute asymmetric response from the ovaries to the VIP on progesterone, testosterone and estradiol serum levels suggesting that in the cyclic rat, each ovary has different sensitivities to VIPergic stimulation which depends on the endocrine status of the animal [20].

To our knowledge, there is no information on the *in vivo* acute effects resulting from ovarian VIP overstimulation in PCOS-EV rats. The aims of the present study were to evaluate the acute effects on progesterone, testosterone, and estradiol serum levels resulting from unilaterally injecting VIP into the ovarian bursa of PCOS-EV rats, with or without unilateral sectioning of the SON. The effects were evaluated one hour after treatment.

Materials and Methods

Experiments were carried out in strict accordance with the Mexican Law of Animal Treatment and Protection Guidelines, and the specifications established in the Mexican Official Standard NOM-062-ZOO-1999. The Committee of the Facultad de Estudios Superiores Zaragoza approved the experimental protocols. All efforts were made to minimize the number of animals used and their suffering.

The study was performed using pre-pubertal female rats of the CIIZ-V strain from our own stock. At birth, the pups were sexed and raised in groups of five females and one male per cage. Rats were housed in acrylic cages under controlled conditions of temperature (22±2 °C) and light (14 h of light/10 h of darkness; with lights on from 05:00 to 19:00 h), with free access to rat chow and tap water. Animals were kept with their dams until day 21 of age, when they were weaned. Once vaginal opening occurred, male rats were withdrawn from the cage. Surgeries were performed between 68 to 72 days of age on vaginal estrus, under ether anesthesia between 10:30 h and 11:30 h. All the animals were sacrificed by decapitation one hour after surgery. Ten rats were used in each experimental group.

Animal Treatment

Ten-day-old rats were injected intramuscularly (i.m) with 0.1 ml of corn oil (vehicle, Vh) or with a single dose of 2 mg of EV (Sigma Chem. Co., St. Luis, Mo. USA) dissolved in 0.1 ml of Vh. The age of first vaginal estrus of these animals was recorded, and estrous cycles were monitored daily by vaginal smears performed at 09:00 h during eight days. When the animals reached 60 days of life, vaginal smears were taken again during eight days. PCOS-EV model rats showed a persistent vaginal estrus, while Vh-injected animals showed a normal vaginal estrous cycle. Once the PCOS-EV and Vh treated animals reached 68 to 72 days old and displayed a vaginal estrus smear, they were assigned to one of the following experimental groups:

Controls

PCOS-EV or Vh treated rats were sacrificed when reached 68 to 72 days old between 11:30 and 12:30 h of estrus day.

Experiment 1: **Acute effects of unilateral sectioning of the SON in PCOS-EV rats**

Unilateral sectioning of the SON was performed following previously described methodology [9, 21]. In brief, Vh and PCOS-EV rats were anesthetized and a dorso-lateral incision was performed on the left or right side, 2 cm below the last rib. The incision affected skin, muscle, and peritoneum. Subsequently, the left or right ovary was exposed and, with the aid of fine forceps, the suspensory ligament enclosing the SON was sectioned at approximately 1 cm from the ovary. The ovary was returned to the abdominal cavity and the wound was sealed.

Experiment 2: **Acute effects of unilateral VIP injection into the ovary of PCOS-EV rats**

VIP injection procedures into the ovarian bursa were performed following previously described methodology [20, 22-25]. In brief, Vh and PCOS-EV treated animals were anesthetized and a dorso-lateral incision was performed as described above. Subsequently, the left or right ovary was exposed and with the aid of a 0.5 ml syringe equipped with a 31G x 8mm gauge needle, were injected 20 µl of VIP 10^{-6} M solution (Sigma Chem. Co., St. Luis, MO, USA) into the left or right ovarian bursa, as previously described by Rosas et al. [20]. To prevent leakage of the VIP solution, and to allow the injected solution to fully cover the ovary, the needle was kept in the bursa for 1 min after injection. Subsequently, the ovary was carefully cleaned, dried, and returned to the abdominal cavity and the wound was sealed.

Experiment 3: **Acute effects of unilateral sectioning of the SON followed by VIPergic stimulation of the denervated ovary in PCOS-EV rats**

As described above, Vh and PCOS-EV animals were anesthetized and a dorso-lateral incision was performed; the SON of the left or right ovary was sectioned and 20 µl of VIP 10^{-6} M were injected into the ovarian bursa of the denervated ovary. Subsequently, the ovary was carefully cleaned, dried, and returned to the abdominal cavity and the wound was sealed.

Autopsy procedures

At the autopsy, the blood of the trunk was collected, allowed to clot at room temperature for 30 min, and centrifuged at 3,000 rpm during 15 minutes. Serum was stored at -20°C, until progesterone, testosterone and estradiol levels were measured using radioimmunoassay (RIA). During autopsy we verified that the ovary was free in the abdominal cavity to confirm that the SON had been properly sectioned.

Hormone assay

Progesterone, testosterone, and estradiol serum levels were measured using RIA with solid-phase kits purchased from Diagnostic Products (Los Angeles, CA, USA). Results are expressed in ng/ml (progesterone) and pg/ml (testosterone and estradiol). The intra- and inter-assay coefficients of variation were 6.58% and 7.42% for progesterone, 7.85% and 8.76% for testosterone and 7.54% and 8.21% for estradiol, respectively.

Statistical analyses

Data on progesterone, testosterone, and estradiol serum levels were analyzed using the Student's t test. A probability value ≤ 0.05 was considered statistically significant.

Results

All rats injected with EV presented characteristic of induced PCOS, including persistent vaginal estrus, anovulation, and the presence of cysts in the ovaries. Testosterone and estradiol serum levels were higher in PCOS-EV animals than in Vh-injected animals. Progesterone levels were similar between PCOS-EV and Vh-treated group (Fig. 1).

Experiment 1: **Acute effects of unilateral sectioning of the SON in PCOS-EV rats**

Compared to the Vh-injected group, sectioning the left SON of PCOS-EV rats resulted in lower estradiol levels (Fig. 1). Sectioning the right SON of PCOS-EV animals resulted in lower estradiol and progesterone levels, and higher testosterone levels than in their corresponding Vh-treated group (Fig. 2).

Experiment 2: **Acute effects of unilateral VIP injection into the ovary of PCOS-EV rats**

In comparison with the Vh-treated group, in PCOS-EV rats the injection of VIP into the left ovary of resulted in lower progesterone and estradiol levels (Fig. 1), while the VIPergic stimulation of the right ovary resulted in lower progesterone and estradiol levels and higher testosterone levels than in (Fig. 2).

Experiment 3: **Acute effects of unilateral sectioning of the SON followed by VIPergic stimulation of the denervated ovary in PCOS-EV rats**

In PCOS-EV rats, injecting VIP into the denervated left ovary resulted in lower testosterone levels than in the corresponding Vh-treated group (Fig. 1). When VIP was injected into the denervated right ovary of PCOS-EV rats, testosterone and estradiol levels were lower than in Vh-treated group (Fig. 2).

In Figure 3 is shown a summary of the progesterone, testosterone and estradiol serum levels in cyclic rats on estrus day or in PCOS rats resulting from the VIP injection into the innervated or denervated ovary.

Discussion

The results obtained in the present study show that in PCOS-EV rats, the SON and the VIP regulate the acute secretion of progesterone, testosterone and estradiol from each ovary in different ways, and that the neural information carried by the SON modulates the VIP effects on steroid hormone secretion.

In vitro studies have shown that steroidogenic response of the ovary of PCOS-EV rat to the gonadotropic and/or neural stimulation is higher than in control animal [6, 17]. The VIPergic stimulation of ovaries obtained from PCOS-EV rats increased androgens and estradiol secretion [17]. In the ovary of PCOS-EV rats, the activation of LH-receptors with human chorionic gonadotropin (hCG), or the activation of β-adrenergic receptors with isoproterenol, resulted in a higher release of androgens [6], despite the low content of β-adrenergic [6] and hCG receptors [26].

In rats on diestrus, the electrical stimulation of the SON increased ovarian progesterone levels through the activation of β-adrenergic receptors [27] while the bilateral sectioning of the SON on the morning of proestrus resulted in an immediate decrease of progesterone plasma levels [28]. In rats on estrus, the left or right SON regulates progesterone secretion in an inhibitory way [29]. Previously we showed that right section of the SON to PCOS-EV rats of 24 days old resulted in lower progesterone levels when the animals reached 90 days of age [9]. The present study yielded similar results, suggesting that the neural information arriving to the ovary via the right SON plays a greater role in regulating progesterone secretion than the information arriving via the left SON. These and other results suggest that the neural information received by the ovaries through the SON is active participants in ovarian steroidogenesis control.

VIP treatment enhanced progesterone release in *in vitro* granulosa cells [30], and in the whole ovary cultures from pre-pubertal and pubertal rats [31]. Previously we showed that the unilateral VIPergic stimulation of the ovary of cyclic rats on estrus does not modify progesterone levels [20]. In the present study we observed that VIPergic overstimulation of any ovary of PCOS-EV rats resulted in lower progesterone levels. This suggests that in the PCOS-EV rat model VIP has an acute inhibitory effect in regulating progesterone secretion, and that such effect depends on the interaction between the neural signals received by the ovary through the SON, as indicated by the absence of inhibitory effects of VIP when the ovaries were previously denervated. This conclusion is supported by studies of Garraza et al. [32], who reported that incubating hemiovaries from rats on diestrus 1 in the presence of NA plus VIP enhanced the inhibitory effect of VIP on progesterone release. In cultured granulosa cells of adult rats on diestrus, VIP's stimulating effect on progesterone secretion is potentiated by phenylonephrine, a α-1 adrenergic agonist, suggesting the simultaneous activation of different peptidergic and adrenergic receptors [33].

Compared to sham-operated group, the section of the left SON to pre-pubertal rats resulted in lower testosterone levels 30 minutes after treatment and higher testosterone levels 60 minutes

after surgery; while the section of the right SON treatment did not modify testosterone levels [21]. The bilateral sectioning of the SON to 4-days old rats resulted in higher androstenedione levels when the animals reached 30 days of age, and turned lower when the animals reached 41 days of age [34]. In PCOS-EV rats, the long lasting effects of unilateral or bilateral sectioning of the SON on testosterone levels [6, 8, 9], suggesting that the neural information carried by the SON to the ovaries stimulates testosterone secretion. In the present study testosterone levels increased after sectioning of the right SON, suggesting that the right SON has an acute inhibitory effect in regulating testosterone secretion. These results support the idea that the role of the SON depends on the time lapse between surgery and autopsy, as previously suggested [21].

VIP stimulates androgen release in ovaries obtained from immature PMSG-injected rats and cyclic rats [17, 31]. Similar effects were observed in cyclic rats on estrus day injected with VIP into the left or right ovarian bursa [20]. In *in vitro* ovaries of EV-treated rats, adding VIP increased the release of androgen [17]. In the present study the effects of unilateral ovarian overstimulation with VIP to PCOS-EV rats depended on which ovary was stimulated, where VIP stimulated testosterone secretion from the right ovary and it had no apparent effect into the left ovary. Such effects depended on the neural signals carried by SON, since in the absence of NA resulting from sectioning the SON, VIP in any denervated ovary has an inhibitory effect on testosterone levels.

In rats, the bilateral sectioning of the SON performed on the morning of proestrus resulted in an immediate drop in estradiol plasma levels [28]. In adult cyclic rats, one hour after sectioning of the left or right SON did not modify estradiol levels, regardless of the day of estrous cycle when the surgery was performed [29]. In PCOS-EV rats the unilateral or bilateral sectioning of the SON resulted in lower estradiol levels [9]. A similar effect was observed in the present study, where the SON appears to regulate estradiol secretion in a stimulating way, supporting

the idea that abnormalities in ovarian steroid secretion are due, at least in part, to an exaggerated sympathetic outflow to the ovary, as proposed by Barria et al. [6].

VIP enhances the activity of the aromatase enzyme complex in *in vitro* fetal ovaries [35], and increases estradiol release from cultured granulosa cells and whole ovaries [17, 30, 31]. Compared to ovaries obtained from control animals on estrus, VIP-induced a greater estradiol release from PCOS-EV rats' ovaries [17]. In the present study, overstimulating the ovaries of PCOS-EV rats with VIP resulted in a lower estradiol secretion. This response appears to depend on the integrity of the SON and suggests that the neural information arriving to the ovaries via the SON is different for each ovary.

The results obtained in the present study support the idea that neural influences arriving to the ovaries through the SON contribute to the steroidogenic alterations observed in the etiology of PCOS rats. The results also suggest that in PCOS-EV rats the neuroendocrine mechanisms regulating ovarian steroid hormones secretion, which depend on the VIPergic and noradrenergic signals of the SON, have different participation in regulating steroid hormone secretion by each ovary. Taken together, these results suggest a functional interaction between neurotransmitters and neuropeptides arriving to the ovary through SON in steroid hormone release regulation.

Acknowledgments

This work was supported by grant UNAM-DGAPA-PAPIIT IN211813. We want to thank for the support given to in the realization of this study to the "Posgrado en Ciencias Biológicas, UNAM" and CONACyT. The revision of the manuscript in English by M Sc Álvaro Domínguez-González is gratefully appreciated.

References

1. Goodarzi, M.O., Dumesic, D.A., Chazenbalk, G., Azziz, R.: Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nat. Rev. Endocrinol. **7**, 219-231 (2011)
2. Matalliotakis, I., Kourtis, A., Koukoura, O., Panidis, D.: Polycystic ovary syndrome: etiology and pathogenesis. Arch. Gynecol. Obstet. **274**, 187-197 (2006)
3. Semenova, I.I.: Adrenergic innervation of ovaries in Stein-Leventhal syndrome. Vestn Akad Med Nauk SSSR. **24**, 58-62 (1969)
4. Heider, U., Pedal, I., Spanel-Borowski, K.: Increase in nerve fibers and loss of mast cells in polycystic and postmenopausal ovaries. Fertil Steril. **75**, 1141-1147 (2001)
5. Brawer, J.R., Munoz, M., Farookhi, R.: Development of the polycystic ovarian condition (PCO) in the estradiol valerate-treated rat. Biol. Reprod. **35**, 647-655 (1986)
6. Barria, A., Leyton, V., Ojeda, S.R., Lara, H.E.: Ovarian steroidal response to gonadotropins and β-adrenergic stimulation is enhanced in polycystic ovary syndrome: role of sympathetic innervation. Endocrinology. **133**, 2696-2703 (1993)
7. Lara, H.E., Dissen, G.A., Leyton, V., Paredes, A., Fuenzalida, H., Fiedler, J.L., Ojeda, S.R.: An increased intraovarian synthesis of nerve growth factor and its low affinity receptor is a principal component of steroid-induced polycystic ovary in the rat. Endocrinology. **141**, 1059-1072 (2000)
8. Rosa-E-Silva, A., Guimaraes, M.A., Padmanabhan, V., Lara, H.E.: Prepubertal administration of estradiol valerate disrupts cyclicity and leads to cystic ovarian morphology during adult life in the rat: role of sympathetic innervation. Endocrinology. **144**, 4289-4297 (2003)
9. Morales-Ledesma, L., Linares, R., Rosas, G., Morán, C., Chavira, R., Cárdenas, M., Domínguez, R.: Unilateral sectioning of the superior ovarian nerve of rats with polycystic ovarian syndrome restores ovulation in the innervated ovary. Reprod. Biol. Endocrinol. **8**, 99 (2010)
10. Lara, H.E., Ferruz, J.L., Luza, S., Bustamante, D.A., Borges, Y., Ojeda, S.R.: Activation of ovarian sympathetic nerves in polycystic ovary syndrome. Endocrinology. **133**, 2690-2695 (1993)

11. Luna, S.L., Neuman, S., Aguilera, J., Brown, D.I., Lara, H.E.: In vivo β-adrenergic blockade by propranolol prevents isoproterenol-induced polycystic ovary in adult rats. Horm. Metab. Res. **44**, 676-681 (2012)

12. Baljet, B., Drukker, J.: The extrinsic innervation of the abdominal organs in the female rat. Acta Anat. (Basel). **104**, 243-267 (1979)

13. Bahr, J.M., Ben-Jonathan, N.: Preovulatory depletion of ovarian catecholamines in the rat. Endocrinology. **108**, 1815-1820 (1981)

14. Aguado, L.I., Ojeda, S.R.: Prepubertal ovarian function is finely regulated by direct adrenergic influences. Role of noradrenergic innervation. Endocrinology. **114**, 1845-1853 (1984)

15. Dees, W.L., Ahmed, C.E., Ojeda, S.R.: Substance P- and vasoactive intestinal peptide-containing fibers reach the ovary by independent routes. Endocrinology. **119**, 638-641 (1986)

16. Dissen, G.A., Paredes, A., Romero, C., Dees, W.L., Ojeda, S.R.: Neural and Neurotrophic Control of Ovarian Development. In: Leung, P.C.K., Adashi E.Y. (eds.) The Ovary, Chapter 1, 2nd edition, pp. 3-23. Elsevier Academic Press, San Diego (2004)

17. Parra, C., Fiedler, J.L., Luna, S.L., Greiner, M., Padmanabhan, V., Lara, H.E.: Participation of vasoactive intestinal polypeptide in ovarian steroids production during the rat estrous cycle and in the development of estradiol valerate-induced polycystic ovary. Reproduction. **133**, 147-154 (2007)

18. Fredericks, C.M., Lundquist, L.E., Mathur, R.S., Ashton, S.H., Landgrebe, S.C.: Effects of vasoactive intestinal peptide upon ovarian steroids, ovum transport, and fertility in the rabbit. Biol. Reprod. **28**, 1052-1060 (1983)

19. Kowalewski, M.P., Dyson, M.T., Boos, A., Stocco, D.M.: Vasoactive intestinal peptide (VIP)-mediated expression and function of steroidogenic acute regulatory protein (StAR) in granulosa cells. Mol. Cell. Endocrinol. **328**, 93-103 (2010)

20. Rosas, G., Ramírez, M.I., Linares, R., Trujillo, A., Domínguez, R., Morales-Ledesma, L.: Asymmetric steroidogenic response by the ovaries to the vasoactive intestinal peptide. Endocrine. **48**, 968-977 (2015)

21. Morales-Ledesma, L., Vieyra, E., Ramírez, D.A., Trujillo, A., Chavira, R., Cárdenas, M., Domínguez, R.: Effects on steroid hormones secretion resulting from the acute stimulation of sectioning the superior ovarian nerve to pre-pubertal rats. Reprod. Biol. Endocrinol. **10**, 88 (2012)

22. Domínguez, R., Cruz, M.E., Morán, C.: Differential effects of ovarian local anaesthesia during pro-oestrus on ovulation by the right or left ovary in normal and hemi-ovariectomized adult rats. J. Reprod. Fertil. **113**, 185-190 (1998)

23. Morán, C., Franco, A., Morán, J.L., Handal, A., Morales, L., Domínguez, R.: Neural activity between ovaries and the prevertebral celiac-superior mesenteric ganglia varies during the estrous cycle of the rat. Endocrine. **26**, 147-152 (2005)

24. Morán, C., Zarate, F., Morán, J.L., Handal, A., Domínguez, R.: Lateralization of the connections of the ovary to the celiac ganglia in juvenile rats. Reprod. Biol. Endocrinol. **7**, 50 (2009)

25. Moran, M.J., Ayala, M.E., Gallegos, E., Romero, J., Chavira, R., Damián-Matsumura, P., Domínguez, R.: Effects of systemic administration or intrabursal injection of serotonin on puberty, first ovulation and follicular development in rats. Reprod. Fertil. Dev. **25**, 1105-1114 (2013)

26. Brawer, J., Richard, M., Farookhi, R.: Pattern of human chorionic gonadotropin binding in the polycystic ovary. Am. J. Obstet. Gynecol. **161**, 474-480 (1989)

27. Weiss, G.K., Dail, W.G., Ratner, A.: Evidence for direct neural control of ovarian steroidogenesis in rats. J. Reprod. Fertil. **65**, 507-511 (1982)

28. Aguado, L.I., Ojeda, S.R.: Ovarian adrenergic nerves play a role in maintaining preovulatory steroid secretion. Endocrinology. **114**, 1944-1946 (1984)

29. Flores, A., Velasco, J., Gallegos, A.I., Mendoza, F.D., Everardo, P.M., Cruz, M.E., Domínguez, R.: Acute effects of unilateral sectioning the superior ovarian nerve of rats with unilateral

ovariectomy on ovarian hormones (progesterone, testosterone and estradiol) levels vary during the estrous cycle. Reprod. Biol. Endocrinol. **9**, 34 (2011)

30. Davoren, J.B., Hsueh, A.J.W.: Vasoactive intestinal peptide: a novel stimulator of steroidogenesis by cultured rat granulosa cells. Biol. Reprod. **33**, 37-52 (1985)

31. Ahmed, C.E., Dees, W.L., Ojeda, S.R.: The immature rat ovary is innervated by vasoactive intestinal peptide (VIP)-containing fibers and responds to VIP with steroid secretion. Endocrinology. **118**, 1682-1689 (1986)

32. Garraza, M.H., Aguado, L.I., De Bortoli, M.A.: *In vitro* effect of neuropeptides on ovary or celiac ganglion affects the release of progesterone from ovaries in the rat. Med. Sci. Monit. **10**, 440-446 (2004)

33. Wasilewska-Dziubiñska, E., Borowiec, M., Chmielowska, M., Woliñska-Witort, E., Baranowska, B.: Alfa 1 Adrenergic Potentiation of Progesterone Accumulation Stimulated by Vasoactive Intestinal Peptide (VIP) and Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) in Cultured Rat Granulosa Cells. Neuro Endocrinol. Lett. **23**, 141-148 (2002)

34. Forneris, M.L., Aguado, L.I.: Neonatal superior ovarian nerve transection disturbs the cyclic activity of the female rats. J. Steroid. Biochem. Mol. Biol. **82**, 75-82 (2002)

35. George, F.W., Ojeda, S.R.: Vasoactive intestinal peptide enhances aromatase activity in the neonatal rat ovary before development of primary follicles or responsiveness to follicle-stimulating hormone. Proc. Natl. Acad. Sci. USA. **84**, 5803-5807 (1987)

Fig. 1 Means ± SEM of progesterone, testosterone and estradiol serum levels in rats that at 10 days old were injected with vehicle (Vh) or EV and at 68 to 72 days old, on vaginal estrus, were submitted to section of the left SON (L-SON), injection of vasoactive intestinal peptide (VIP) into the left (L) ovarian bursa or to L-SON plus VIP injection into the denervated ovary. Animals were sacrificed 1 h after treatments. * $p<0.05$ vs. its corresponding Vh group (Student's t test)

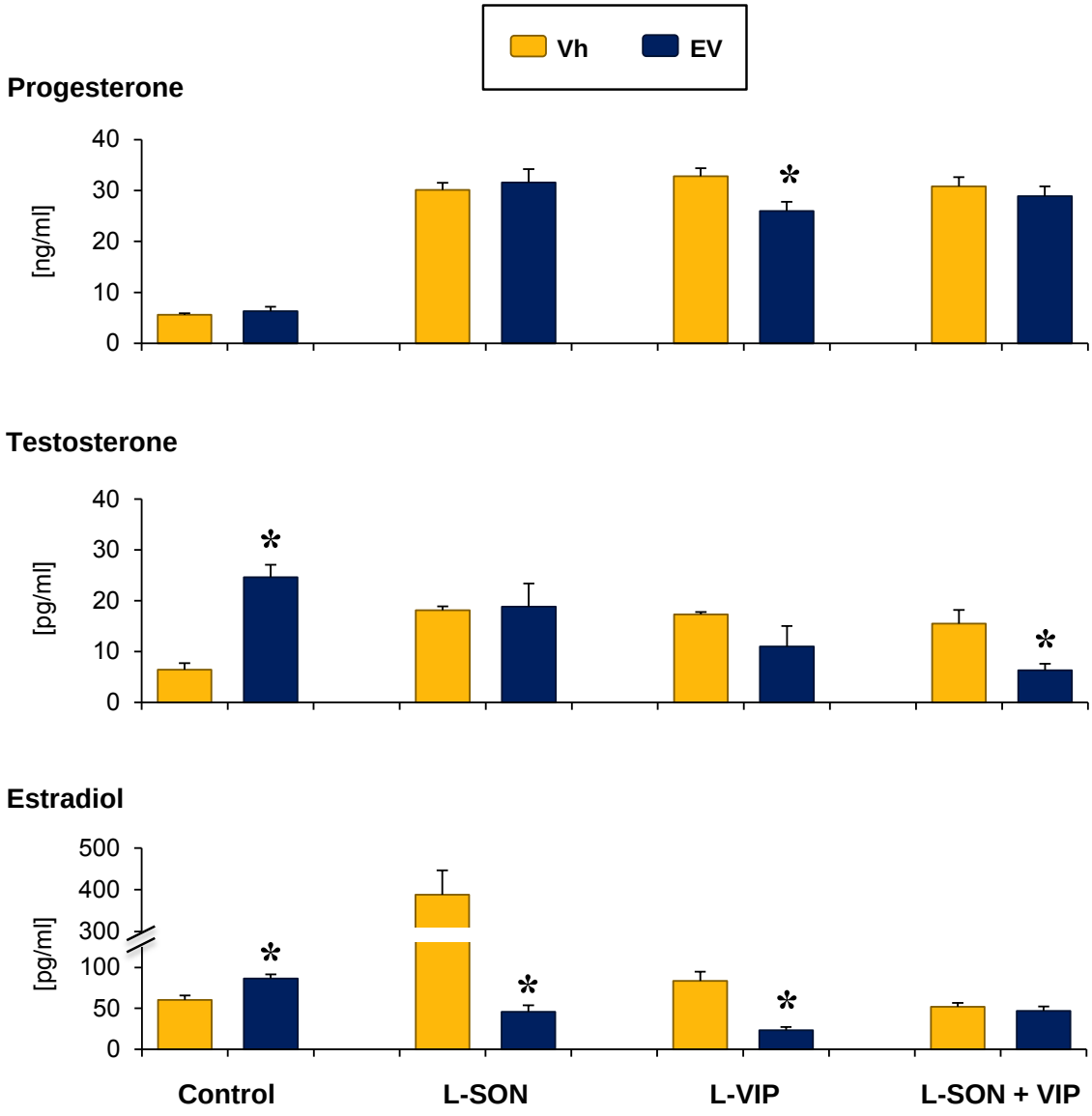


Fig. 2 Means ± SEM of progesterone, testosterone and estradiol serum levels in rats that at 10 days old were injected with vehicle (Vh) or EV and at 68 to 72 days old, on vaginal estrus, were submitted to section of the right SON (R-SON), injection of vasoactive intestinal peptide (VIP) into the right (R) ovarian bursa or to R-SON plus VIP injection into the denervated ovary. Animals were sacrificed 1 h after treatments. * $p<0.05$ vs. its corresponding Vh group (Student's t test)

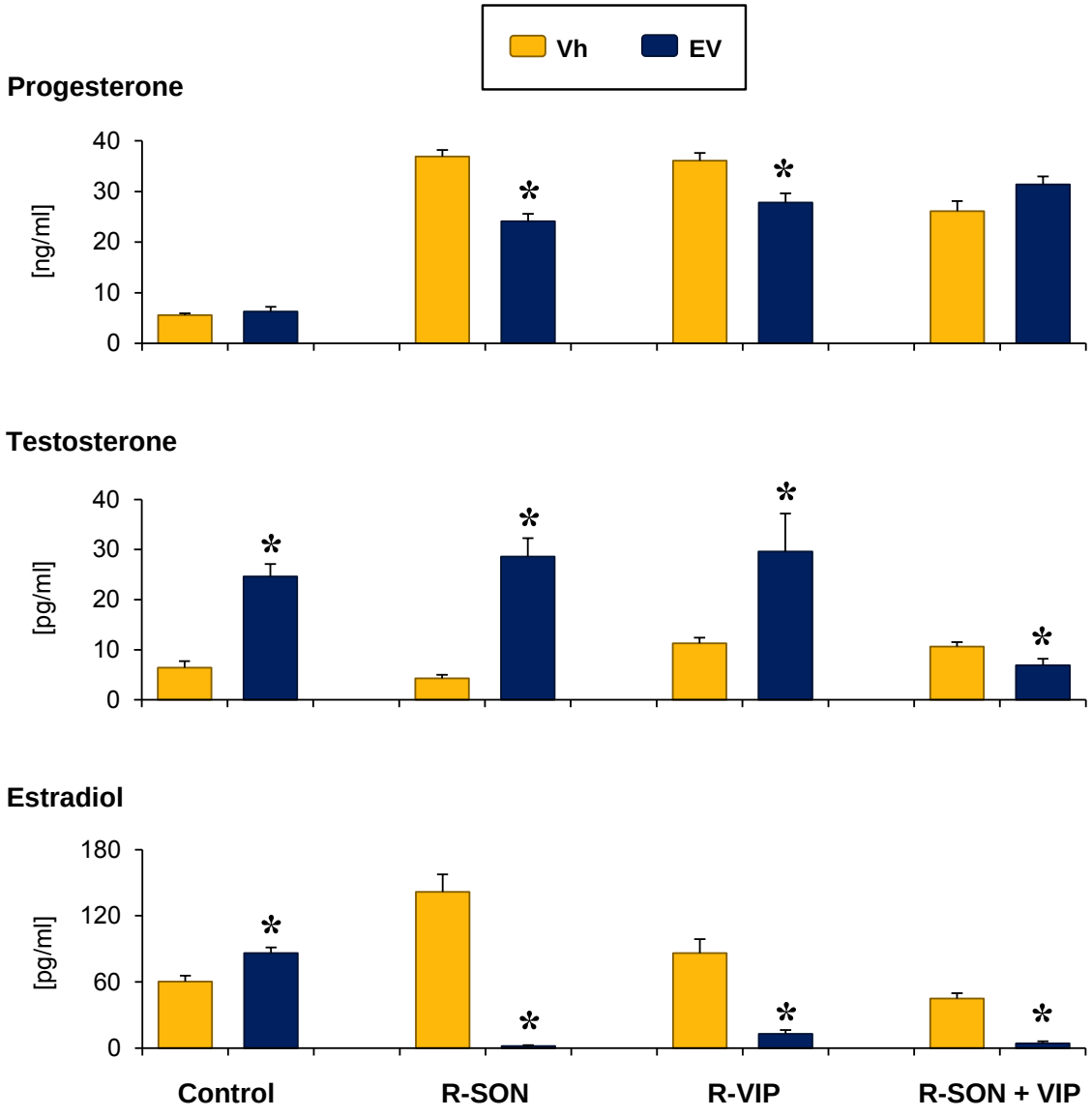
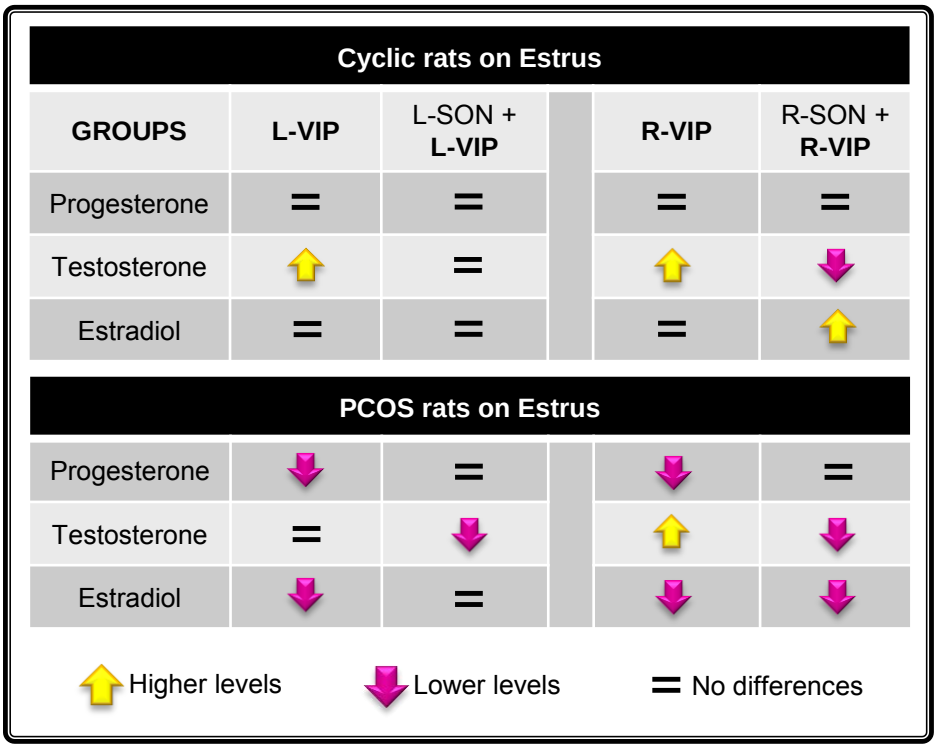


Fig. 3 Summary of effects of VIP injection into the innervated or denervated ovary of cyclic rats on estrus or PCOS rats. Progesterone, testosterone and estradiol serum levels compared to theirs respective vehicle groups. Progesterone, testosterone and estradiol serum levels in cyclic rats resulting from the unilateral VIP injection into the left (L-VIP) or right (R-VIP) ovarian bursa (based on data published by Rosas et al. [2015]) or from the section of the left (L-SON) or right (R-SON) superior ovarian nerve and injected with VIP into the denervated ovary.



DISCUSIÓN GENERAL

Los resultados del presente estudio muestran que en la rata adulta cíclica cada ovario tiene diferente sensibilidad al estímulo VIPérgico que depende del ambiente endocrino del animal. En la rata con SOPQ el VIP estimula la síntesis de testosterona sólo en el ovario derecho, lo que sugiere que cada ovario responde de manera diferente frente a un mismo estímulo. Las señales nerviosas que ingresan al ovario por el NOS modulan de manera diferente la respuesta esteroidogénica del ovario al estímulo VIPérgico en el animal con SOPQ que en el animal cíclico.

El ovario recibe inervación simpática a través del NOS, por el que transcurren fibras que contienen NA, NPY y VIP (Baljet y Drukker, 1979; Lawrence y Burden, 1980; Bahr y Ben-Jonathan, 1981; Dees y col., 1986; Schulstea y col., 1992). Diversas evidencias indican que el VIP estimula la secreción de hormonas esteroides (Fredericks y col., 1983; Davoren y Hsueh, 1985; Ahmed y col., 1986; Johnson y col., 1994; Parra y col., 2007), lo cual se explica por el incremento en la actividad de proteínas acarreadoras como la StAR y de enzimas como la 3β-HSD y las aromatasas, que participan en la síntesis de hormonas esteroides en el ovario (Davoren y Hsueh, 1985; George y Ojeda, 1987; Kowalewski y col., 2010). Según Garraza y col. (2004) los efectos del VIP sobre la secreción de progesterona dependen del día del ciclo estral lo cual concuerda con los resultados de nuestro estudio.

Estudios *in vitro* muestran que los efectos del VIP sobre la secreción de hormonas esteroides por células de la granulosa aisladas o el ovario completo dependen del lapso entre el estímulo y la evaluación de la secreción hormonal (Davoren y Hsueh, 1985; Garraza y col., 1994; Wasilewska-Dziubiñska y col., 2002). En cultivo de células de la granulosa, la inhibición de la actividad enzimática de la P450scc impide el efecto estimulante del VIP sobre la secreción de progesterona, y un efecto similar se observa si se inhibe la síntesis del ARNm con actinomicina D con lo que se bloquea la síntesis de novo de la P450scc (Wasilewska-Dziubiñska y col., 2002). En el presente estudio los efectos de la estimulación VIPérgica del ovario sobre la secreción de

hormonas esteroides dependen del tiempo transcurrido entre el estímulo y la evaluación de sus efectos. Tomando en conjunto los resultados del grupo de Wasilewska-Dziubiñska (2002) y los nuestros proponemos que a corto plazo (1 hora) el VIP estimula la fosforilación y activación de enzimas pre-existentes, mientras que a largo plazo (24 horas) estimula además la síntesis de nuevas enzimas.

En el modelo de la rata cíclica cada ovario tiene diferente sensibilidad al estímulo VIPérgico que cambia ante la falta del NOS. Esto nos permite sugerir que los efectos del VIP son modulados por las señales nerviosas de naturaleza simpática que reciben los ovarios a través del NOS, aunque no se descarta que tanto el NV como el NPO participen en la regulación de los efectos del VIP en el ovario. Se ha mostrado que la lesión izquierda, derecha o bilateral del área preóptica hipotalámica anterior incrementa la concentración de VIP en el ovario izquierdo, lo que sugiere que el control hipotalámico de VIP en el ovario es asimétrico (Advis y col., 1989). Otra posibilidad es que los ovarios responden de manera diferente al estímulo VIPérgico y en ese caso la asimetría estaría a nivel ovárico posiblemente como resultado de una diferente expresión de los receptores a VIP.

En el presente estudio se confirma que la inyección de VE en la etapa infantil resulta en el desarrollo del SOPQ, caracterizado por la alteración del ciclo estral, falta de ovulación, presencia de quistes foliculares y altas concentraciones de testosterona y estradiol, tal como ha sido reportado previamente (Rosas, 2006; Rosa-E-Silva y col., 2003; Morales-Ledesma y col., 2010).

En las ratas con SOPQ incrementa la actividad de las fibras simpáticas del NOS y la concentración ovárica de NA (Lara y col., 1993). La sección bilateral del NOS en ratas con SOPQ resulta a largo plazo (10 ó 21 días) en una menor concentración de NA ovárica, normalización del ciclo estral, de la ovulación y en menores concentraciones de andrógenos y estradiol (Barria y col., 1993; Rosa-E-Silva y col., 2003). Previamente mostramos que a los 60 días de realizada la sección unilateral del NOS en ratas con SOPQ se normalizan las concentraciones de testosterona y estradiol en suero y se restaura la ovulación en el ovario inervado, por lo que se

sugirió que en el SOPQ además de la hiperactividad NAérgica existen otros factores que contribuyen al desarrollo de la patología (Morales-Ledesma y col., 2010), como pueden ser el VIP (Rosas, 2011; Ramírez, 2014; presente estudio), y las señales nerviosas que llegan al ovario a través del NPO (Díaz, 2013) y del NV (Linares y col., 2013).

La testosterona en uno de los precursores del estradiol, por lo que en varias circunstancias se ha observado que la disminución en la concentración de testosterona es acompañada de un aumento significativo en la concentración de estradiol debido a su biotransformación (Strauss y Williams, 2009; Domínguez y col., 2011; Miller y Auchus, 2011). En el presente estudio observamos que una hora después de la sección unilateral del NOS en ratas con SOPQ disminuye la concentración de estradiol sin que se modifique la de testosterona, lo que nos sugiere que la inervación que llega por el NOS regula de manera estimulante la actividad de las aromatasas. Esta idea se puede apoyar en el hecho de que en un sistema *ex vivo* ganglio celiaco-NOS-ovario de ratas en proestro, la adición de NA en el compartimiento ganglionar resultó el incremento del ARNm de las aromatasas (Delgado y col., 2010).

En los animales con SOPQ incrementa la concentración de VIP en el ovario. *In vitro*, la adición del péptido a ovarios de animales con el síndrome aumenta la secreción de andrógenos (Parra y col., 2007), un efecto semejante se observó en el presente estudio por la estimulación VIPérgica del ovario derecho de animales con SOPQ.

Previamente mostramos que 24 horas después de la estimulación VIPérgica del ovario izquierdo denervado de ratas con SOPQ incrementa la secreción de testosterona, contrario a lo observado en el ovario derecho denervado (Rosas, 2011). En la rata con SOPQ, el bloqueo de los receptores VIPérgicos del ovario izquierdo resulta a los 30 minutos en una mayor concentración ovárica de NA que se acompaña del incremento de la concentración de testosterona en suero. Estos efectos no se observan cuando se bloquean los receptores del ovario derecho (Ramírez, 2014). Los resultados del presente estudio muestran que en cualquiera de los ovarios denervados el VIP regula de manera inhibitoria la secreción aguda de

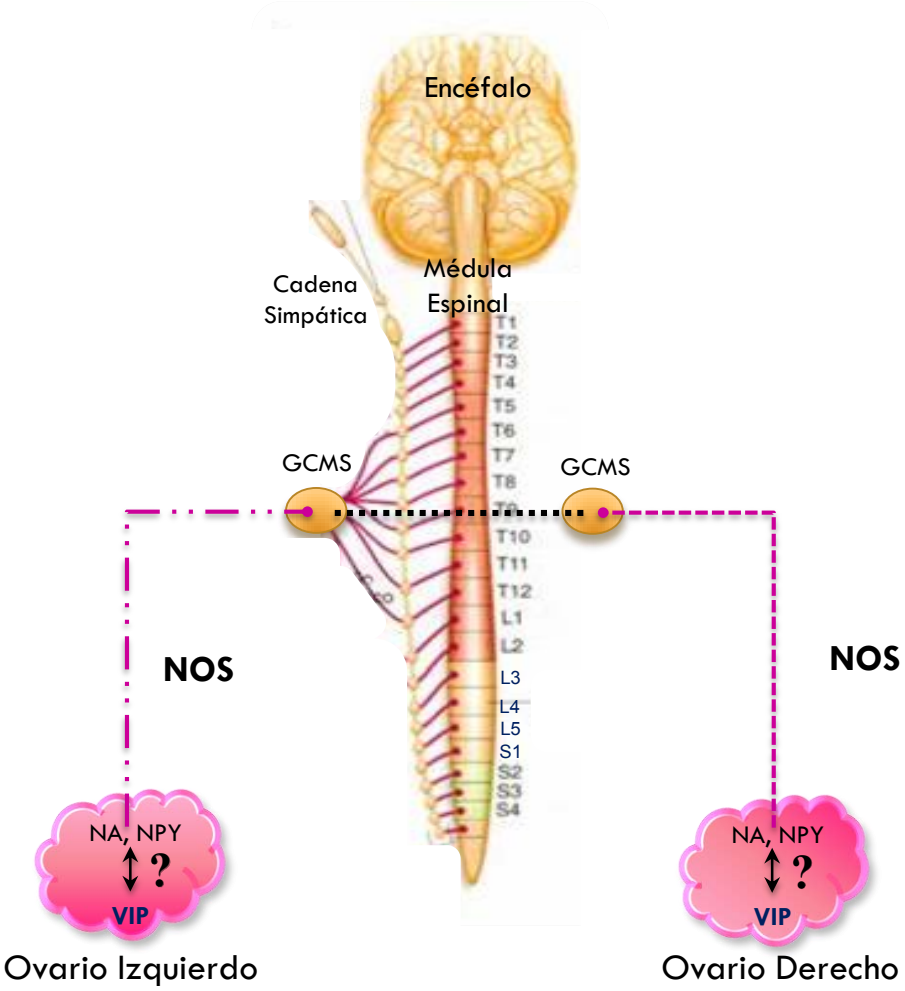
testosterona, por lo que sugerimos que en la rata con SOPQ los efectos del VIP varían en función del tiempo y dependen de su interacción con las señales nerviosas que ingresan al ovario a través del NOS.

In vitro el VIP incrementa la actividad de las aromatasas en ovarios fetales de ratas (George y Ojeda, 1987) así como la secreción de estradiol por ovarios de ratas cíclicas o con SOPQ (Parra y col., 2007). En el presente estudio, la estimulación del sistema VIPérgico de animales cíclicos o con SOPQ resultó en una menor concentración de estradiol en suero. Estas diferencias ponen de manifiesto que *in vivo*, los ovarios reciben diferentes señales endocrinas y nerviosas que regulan sus funciones, lo que no existe en los estudios *in vitro*.

Con la información disponible hasta el momento no se puede discernir cuáles son los factores que explican las diferencias observadas en la respuesta de los ovarios de ratas cíclicas en estro y de ratas con SOPQ, frente al estímulo VIPérgico y su relación con la inervación NAérgica que llega por el NOS. Algunos de los factores que pudieran estar involucrados serían el tipo y número de receptores a VIP presentes en los ovarios de los animales en estro y en ratas con SOPQ, la sensibilidad de los receptores, así como las vías de señalización celular estimuladas por la unión del VIP a cada uno de sus receptores.

En conjunto, los resultados del presente estudio muestran que en los ovarios, los mecanismos neuroendocrinos que regulan la secreción aguda de hormonas esteroides dependen de las señales VIPérgicas, NAérgicas y quizás las NPYérgicas que ingresan al ovario a través del NOS y que la interacción de estas señales nerviosas modula en diferentes formas la respuesta de cada ovario al estímulo VIPérgico. Asimismo, muestran que la interacción funcional entre los neurotransmisores y neuropéptidos que transcurren por el NOS es distinta en un animal con la patología del SOPQ que en uno que no la presenta.

Ratas cíclicas en la etapa del diestro 2



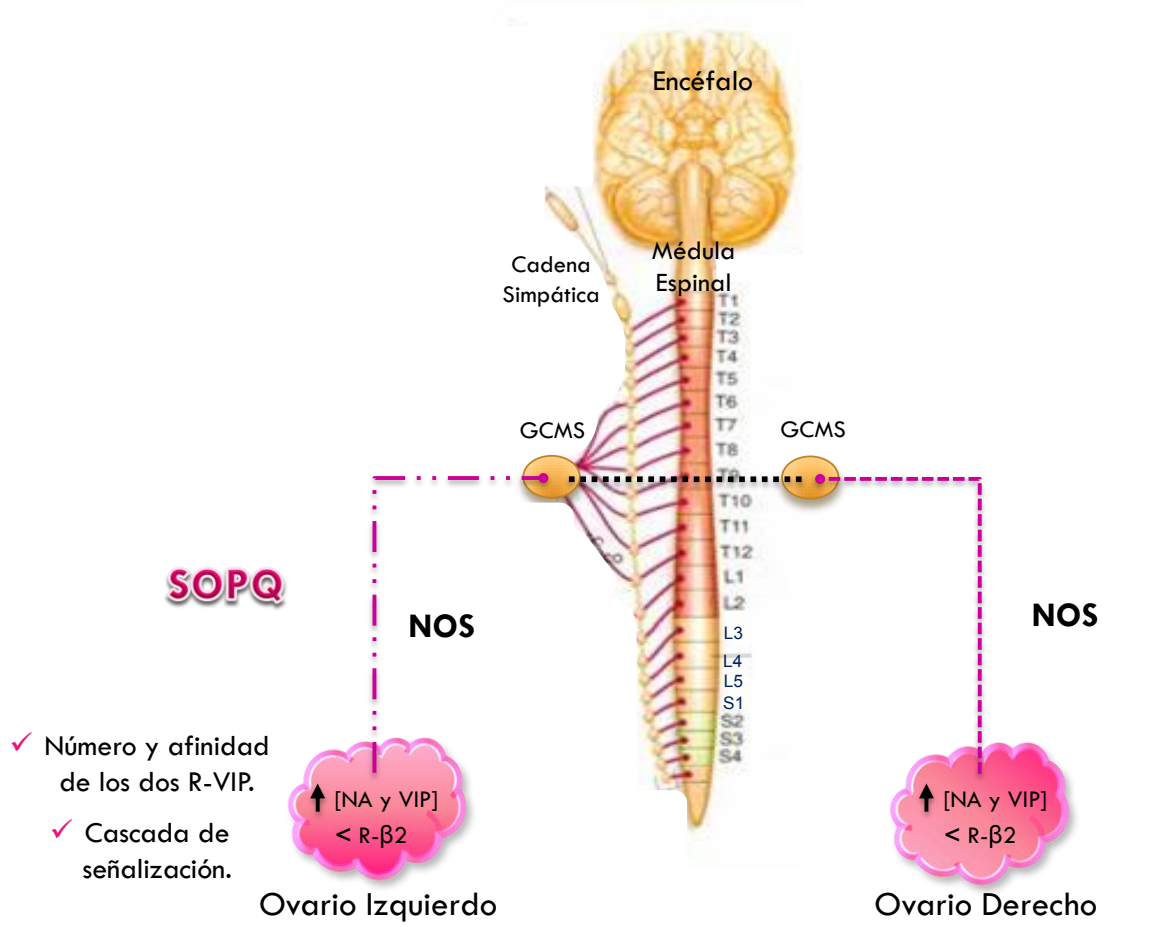
	VIP OI	SNOI + VIP OI	SNOD + VIP OI
P4	=	=	=
T	↑	=	↓
E2	↓	↑	↑↑

	VIP OD	SNOD + VIP OD	SNOI + VIP OD
P4	↑	=	↑
T	=	=	=
E2	=	↑	↓

Figura 1. Efectos de la estimulación VIPérgica del ovario de ratas cíclicas en diestro 2 con o sin previa sección unilateral del NOS. **GCMS**, ganglio celiaco mesentérico superior; **NOS**, nervio ovárico superior; **NA**, noradrenalina; **NPY**, neuropéptido Y; **VIP**, péptido intestinal vasoactivo; **P4**, progesterona; **T**, testosterona; **E2**, estradiol; **OI**, ovario izquierdo; **OD**, ovario derecho; **SNOI**, sección del NOS izquierdo; **SNOD**, sección del NOS derecho. Las flechas indican disminuciones (flecha azul) o aumentos (flecha rosa) en la concentración de hormonas esteroides, mientras que los signos de igual indican que no hubo cambios significativos.

En la figura 1 se observa que la secreción aguda de hormonas esteroides por el ovario izquierdo y derecho de ratas en diestro 2 frente al estímulo VIPérgico es asimétrica, lo que nos sugiere que cada ovario tiene diferente sensibilidad al VIP. La respuesta diferencial de los ovarios al péptido es regulada por las señales nerviosas que ingresan al ovario a través del NOS, donde ante la falta de esta información simpática, el VIP regula de manera estimulante la secreción de estradiol en ambos ovarios denervados. La respuesta del ovario estimulado con VIP cambia cuando el ovario contralateral es previamente denervado, por lo que proponemos que las señales del NOS izquierdo y derecho son indispensables para que cada ovario traduzca la señal VIPérgica, además de que estos resultados apoyan la existencia de una comunicación nerviosa entre los ovarios dada a través del NOS.

Ratas cíclicas en estro y ratas con SOPQ



	ESTRO		SOPQ	
	VIP OI	SNOI + VIP	VIP OI	SNOI + VIP
P4	=	=	↓	=
T	↑	=	=	↓
E2	=	=	↓	=

	ESTRO		SOPQ	
	VIP OD	SNOD + VIP	VIP OD	SNOD + VIP
	=	=	↓	=
	↑	↓	↑	↓
	=	↑	↓	↓

Figura 2. Efectos de la estimulación VIPérgica del ovario de ratas cíclicas en estro con o sin previa sección unilateral del NOS, en comparación a los efectos en ratas con SOPQ. GCMS, ganglio celiaco mesentérico superior; **NOS**, nervio ovárico superior; **NA**, noradrenalina; **NPY**, neuropéptido Y; **VIP**, péptido intestinal vasoactivo; **R**, receptor; **SOPQ**, síndrome del ovario poliquístico; **P4**, progesterona; **T**, testosterona; **E2**, estradiol; **OI**, ovario izquierdo; **OD**, ovario derecho; **SNOI**, sección del NOS izquierdo; **SNOD**, sección del NOS derecho. Las flechas indican disminuciones o aumentos en la concentración de hormonas esteroides de ratas cíclicas en estro (flecha rosa) o con SOPQ (flecha azul), mientras que los signos de igual indican que no hubo cambios significativos.

En la figura 2 se muestra que en el día del estro, el VIP regula de manera estimulante la secreción de testosterona en cualquiera de los ovarios, mientras que en la rata con SOPQ este efecto únicamente lo tiene en el ovario derecho. El papel del VIP en ambos modelos experimentales depende de la integridad del NOS, por lo que sugerimos que la NA, el VIP y el NPY que ingresan al ovario a través del NOS interactúan de manera diferente sobre la regulación de la secreción de hormonas esteroides. Es posible que la mayor afluencia de VIP y de NA al OPQ, así como la disminución del número de receptores β-adrenérgicos y el incremento de su sensibilidad, parámetros que han sido descritos en la literatura, sean determinantes en la diferente respuesta al VIP que tienen los ovarios de ratas con SOPQ que de ratas en estro. Con estos resultados surge la interrogante de si la respuesta del OPQ al VIP se debe a una modificación en el número y afinidad de los receptores a VIP o si es la cascada de señalización activada por cada receptor la que se encuentre alterada. Estos aspectos requieren ser estudiados a detalle.

CONCLUSIONES GENERALES

En el modelo de la rata adulta cíclica:

- La distensión de la bursa ovárica izquierda o derecha por efecto de la inyección de solución salina incrementó la concentración de progesterona en suero, mientras que sus efectos sobre testosterona o estradiol dependen del ovario en estudio y del día del ciclo estral.

- La tasa de secreción de hormonas esteroides en respuesta a la estimulación del sistema VIPérgico del ovario izquierdo o derecho dependen de la hormona analizada y del estado endocrino de la rata.

- Las señales nerviosas que recibe cada ovario a través del NOS modulan de manera estimulante o inhibitoria la sensibilidad del ovario al estímulo con VIP y su participación varía en función del día del ciclo estral. Los resultados apoyan la hipótesis de la existencia de una comunicación nerviosa entre los ovarios a través del NOS, cuya información parece ser indispensable para que el ovario traduzca la señal VIPérgica.

En el modelo de la rata adulta con SOPQ:

- La información del NOS regula de manera diferencial la secreción de hormonas esteroides por cada ovario. Es estimulante en el ovario izquierdo sobre la secreción de estradiol, mientras que en el ovario derecho estimula la secreción de progesterona y estradiol e inhibe la de testosterona.

- En ambos ovarios, el VIP modula de manera inhibitoria la secreción de progesterona y estradiol. En el ovario derecho, el VIP tiene un efecto estimulante sobre la secreción de testosterona.

- En cualquiera de los ovarios denervados el VIP tiene un efecto inhibitorio sobre la secreción de testosterona.

REFERENCIAS GENERALES

- Abbott DH, Barnett DK, Bruns CM, Dumesic DA. (2005) Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? Hum Reprod Update. 11: 357-374.

- Abbott DH, Barnett DK, Levine JE, Padmanabhan V, Dumesic DA, Jacoris S, Tarantal AF. (2008) Endocrine antecedents of polycystic ovary syndrome in fetal and infant prenatally androgenized female rhesus monkeys. Biol Reprod. 79: 154-163.

- Abbott DH, Tarantal AF, Dumesic DA. (2009) Fetal, infant, adolescent and adult phenotypes of polycystic ovary syndrome in prenatally androgenized female rhesus monkeys. Am J Primatol. 71: 776-784.

- Adashi EY, Hsueh AJ. (1981) Stimulation of beta 2-adrenergic responsiveness by follicle-stimulating hormone in rat granulosa cells in vitro and in vivo. Endocrinology. 108: 2170-2178.

- Advis JP, Ahmed CE, Ojeda SR. (1989) Direct hypothalamic control of vasoactive intestinal peptide (VIP) levels in the developing rat ovary. Brain Serearch Bulletin. 22: 605-610.

- Aguado LI, Ojeda SR. (1984a) Ovarian adrenergic nerves play a role in maintaining preovulatory steroid secretion. Endocrinology. 114: 1944-1946.

- Aguado LI, Ojeda SR. (1984b) Prepubertal ovarian function is finely regulated by direct adrenergic influences. Role of noradrenergic inervation. Endocrinology. 114: 1845-1853.

- Aguado LI, Petrovic SL, Ojeda SR. (1982) Ovarian β-adrenergic receptors during the onset of puberty: characterization, distribution and coupling to steroidogenic responses. Endocrinology. 110: 1124-1132.

- Ahmed CE, Dees WL, Ojeda SR. (1986) The immature rat ovary is innervated by vasoactive intestinal peptide (VIP)-containing fibers and responds to VIP with steroid secretion. Endocrinology. 118: 1682-1689.

- Anderson LA, McTernan PG, Harte AL, Barnett AH, Kumar S. (2002) The regulation of HSL and LPL expression by DHT and flutamide in human subcutaneous adipose tissue. Diabetes Obes Metab. 4: 209-213.

- Anesetti G, Lombide P, Chávez-Genaro R. (2009) Prepubertal estrogen exposure modifies neurotrophin receptor expression in celiac neurons and alters ovarian innervation. Auton Neurosci. 145: 35-43.

- Arner P. (2005) Effects of testosterone on fat cell lipolysis. Species differences and possible role in polycystic ovarian syndrome. Biochimie. 87: 39-43.

- Bahr JM, Ben-Jonathan N. (1981) Preovulatory depletion of ovarian catecholamines in the rat. Endocrinology. 108: 1815-1820.

- Baldani DP, Skrgatic L, Ougouag R. (2015) Polycystic Ovary Syndrome: important underrecognised cardiometabolic risk factor in reproductive-age women. Int J Endocrinol. doi: 10.1155/2015/786362.

- Baljet B, Drukker J. (1979) The extrinsic innervation of the abdominal organs in the female rat. Acta Anat. (Basel). 104: 243-267.

- Baljet B, Drukker J. (1980) The extrinsic innervation of pelvic organ in the female rat. Acta Anat. 107: 241-267.

- Barra R, Cruz G, Mayerhofer A, Paredes A, Lara HE. (2014) Maternal sympathetic stress impairs follicular development and puberty of the offspring. Reproduction. 148: 137-145.

- Barria A, Leyton V, Ojeda SR, Lara HE. (1993) Ovarian steroidal response to gonadotropins and β-adrenergic stimulation is enhanced in polycystic ovary syndrome: role of sympathetic innervation. Endocrinology. 133: 2696-2703.

- Battaglia C, Regnani G, Mancini F, Iughetti L, Flamigni C, Venturoli S. (2002) Polycystic ovaries in childhood: a common finding in daughters of PCOS patients. A pilot study. Hum Reprod. 17: 771-776.

- Beloosesky R, Gold R, Almog B, Sasson R, Dantes A, Land-Bracha A, Hirsh L, Itskovitz-Eldor J, Lessing JB, Homburg R, Amsterdam A. (2004) Induction of polycystic ovary by

testosterone in immature female rats: modulation of apoptosis and attenuation of glucose/insulin ratio. Int J Mol Med. 14: 207-215.

- Bernuci MP, Szawka RE, Helena CV, Leite CM, Lara HE, Anselmo-Franci JA. (2008) Locus coeruleus mediates cold stressinduced polycystic ovary in rats. Endocrinology 149: 2907-2916.

- Blank SK, McCartney CR, Marshall JC. (2006) The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. Hum Reprod Update. 12: 351-361.

- Brawer JR, Munoz M, Farookhi R. (1986) Development of the polycystic ovarian condition (PCO) in the estradiol valerate-treated rat. Biol Reprod. 35: 647-655.

- Bremer AA. (2010) Polycystic ovary syndrome in the pediatric population. Metab Syndr Relat Disord. 8: 375-394.

- Bronstein J, Tawdekar S, Liu Y, Pawelczak M, David R, Shah B. (2011). Age of onset of polycystic ovarian syndrome in girls may be earlier than previously thought. J Pediatr Adolesc Gynecol. 24: 15-20.

- Burden HW. (1978) Ovarian innervation. En: Jones R.E. (ed.) The vertebrate ovary comparative biology. Plenium Press. New York. pp. 615-628.

- Burden HW. (1985) The adrenergic innervation of mammalian ovaries. En: Ben-Jonathan N, Barh JM, Weiner RI. (Eds.) Catecholamines as hormone regulator. Raven Press. New York. pp. 261-278.

- Burden HW. 1972. Adrenergic innervation in ovaries of the rats and guinea pigs. Am J Anat. 133: 455-461.

- Carmina E, Koyama T, Chang L, Stanczyk FZ, Lobo RA. (1992) Does ethnicity influence the prevalence of adrenal hyperandrogenism and insulin resistance in polycystic ovary syndrome? Am J Obstet Gynecol. 167: 1807-1812.

- Chen N, Li Y, Wang W, Ma Y, Yang D, Zhang Q. (2013) Vasoactive intestinal peptide can promote the development of neonatal rat primordial follicles during in vitro culture. Biol Reprod. 88: 12. doi: 10.1095/biolreprod.111.098335.

- Chittenden BG, Fullerton G, Maheshwari A, Bhattacharya S. (2009) Polycystic ovary syndrome and the risk of gynaecological cancer: a systematic review. Reprod Biomed. 19: 398-405.

- Chrousos GP, Gold PW. (1992) The concepts of stress system disorders: overview of behavioral and physical homeostasis. JAMA. 267: 1244-1252.

- Ciaraldi TP, el-Roeiy A, Madar Z, Reichart D, Olefsky JM, Yen SS. (1992) Cellular mechanisms of insulin resistance in polycystic ovary syndrome. J Clin Endocrinol Metab. 75: 577-583.

- Conway GS, Avey C, Rumsby G. (1994) The tyrosine kinase domain of the insulin receptor gene is normal in women with hyperinsulinaemia and polycystic ovary syndrome. Hum Reprod. 9: 1681-1683.

- Da Silva BB, Lopes-Costa PV, Dos Santos AR, Gomes Pires C, Soares Borges C, Gontijo JA. (2009) Evaluation of Ki-67 antigen expression in the zona reticularis of the adrenal cortex of female rats in persistent estrus. Hum Reprod. 24: 705-709.

- Da Silva BB, Lopes-Costa PV, Rosal MA, Pires CG, dos Santos LG, Gontijo JA, Alencar AP, de Jesus Simoés M. (2007) Morphological and morphometric analysis of the adrenal cortex of androgenized female rats. Gynecol Obstet Invest. 64: 44-48.

- Davoren JB, Hsueh AJW. (1985) Vasoactive Intestinal Peptide: a novel stimulator of steroidogenesis by cultured rat granulosa cells. Biol Reprod. 33: 37-52.

- Dees WL, Ahmed CE, Ojeda SR. (1986) Substance P and vasoactive intestinal peptide-containing fibers reach the ovary by independent routes. Endocrinology. 119: 638-641.

- Delgado SM, Escudero CG, Casais M, Gordillo M, Anzulovich AC, Sosa Z, Rastrilla AM. (2010) Ovaric physiology in the first oestral cycle: influence of noradrenergic and cholinergic neural stimuli from coeliac ganglion. Steroids. 75: 685-694.

- Demissie M, Lazic M, Foecking EM, Aird F, Andrea Dunaif, Levine JE. (2008) Transient prenatal androgen exposure produces metabolic syndrome in adult female rats. Am J Physiol Endocrinol Metab. 295: E262-E268. doi: 10.1152/ajpendo.90208.2008.

- DeUgarte CM, Bartolucci AA, Azziz R. (2005) Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertil Steril. 83: 1454-1460.

- Diamanti-Kandarakis E, Dunaif A. (2012) Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocrine Reviews. 33: 981-1030.

- Diamanti-Kandarakis E. (2008) Polycystic ovarian syndrome: pathophysiology, molecular aspects and clinical implications. Expert Rev Mol Med. 10:e3. doi: 10.1017/S1462399408000598.

- Díaz RJA. (2013) Posible comunicación interovarica vía el plexo ovárico en la persistencia del síndrome del ovario poliquístico: la rata como modelo de estudio. Tesis de Maestría. UNAM, FES Zaragoza.

- Dicken C, Menke M, Neal-Perry G. (2010) The hypothalamic-pituitary-ovarian axis. Santoro NF, Neal-Perry G (Eds.) Amenorrhea: a case-based, clinical guide, contemporary endocrinology. Springer Science+Business Media. Cap. 1. pp 1-19. doi: 10.1007/978-1-60327-864-5_1.

- Dissen G, Ojeda SR. (1999) Ovarian innervation. Encyclopedia of Reproduccion. Academic Press. USA. 3: 583-589.

- Dissen GA, Paredes A, Romero C, Dees WL, Ojeda SR. (2004) Neural and neurotrophic control of ovarian development. In: Leung PCK, Adashi EY. (eds.) The Ovary, Elsevier Academic Press, San Diego, Cap. 1, segunda edición, pp. 3-23.

- Domínguez R, Chávez R, Cruz ME. (1991) La regulación del crecimiento y del desarrollo del folículo ovárico. En: Tópicos selectos de biología de la reproducción. Miguel Ángel Porrúa. México. pp. 161-192.

- Domínguez R, Cruz-Morales SE. (2011) The ovarian innervation participates in the regulation of ovarian functions. Endocrinol Metabol Syndrome. S4:001. doi:10.4172/2161-1017.S4-001.

- Domínguez R, Flores A, Cruz-Morales SE. (2011) Hormonal and neural mechanisms regulating hormone steroids secretion. In: Hassan, A. (ed.) Steroids Basic Science. Publisher In Tech. Croacia, Rijeka. Cap. 1, pp. 3-34. ISBN 978-953-307-866-3.

- Dorfman M, Arancibia S, Fiedler JL, Lara HE. (2003) Chronic intermittent cold stress activates ovarian sympathetic nerves and modifies ovarian follicular development in the rat. Biol Reprod. 68: 2038-2043.

- Dumitrescu R, Mehedintu C, Briceag I, Purcarea VL, Hudita D. (2015) The polycystic ovary syndrome: an update on metabolic and hormonal mechanisms. J Med Life. 8: 142-145.

- Dunaif A, Segal KR, Futterweit W and Dobrjansky A. (1989). Profound Peripheral Insulin Resistance, independent of Obesity, in Polycystic Ovary Syndrome. Diabetes. 38: 1165-1174.

- Dunaif A, Xia J, Book C-B, Schenker E, Tang Z. (1995) Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. A potential mechanism for insulin resistance in the polycystic ovary syndrome. J Clin Invest. 96: 801-810.

- Dyer C, Erickson G. (1985) Norepinephrine amplifies human chorionic gonadotropin-stimulated androgen biosynthesis by ovarian theca-interstitial cells. Endocrinology. 116: 1645-1652.

- Eagleson CA, Gingrich MB, Pastor CL, Arora TK, Burt CM, Evans WS, Marshall JC. (2000) Polycystic ovarian syndrome: evidence that flutamide restores sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. J Clin Endocrinol Metab. 85: 4047-4052.

- Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. (1999). Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care. 22: 141-146.

- Erickson GF, Magofin DA, Dyer CA, Hofeditz C. (1985) The ovarian androgen-producing cells: a review of structure/function relationships. Endocrin Rev. 6: 371-399.

- Fica S, Albu A, Constantin M, Dobri GA, Davila C. (2008) Insulin resistance and fertility in polycystic ovary syndrome. J Med Life. 1: 415-422.

- Flaws JA, Desanti A, Tilly KI, Javid RO, Kugu K, Johnson AL, Hirshfield AN, Tilly JL. (1995) Vasoactive Intestinal Peptide-mediated suppression of apoptosis in the ovary: potential mechanisms of action and evidence of a conserved antiatretogenic role through evolution. Endocrinology. 136: 4351-4359.

- Flores A, Velasco J, Gallegos AI, Mendoza FD, Everardo PM, Cruz ME, Domínguez R. (2011) Acute effects of unilateral sectioning the superior ovarian nerve of rats with unilateral ovariectomy on ovarian hormones (progesterone, testosterone and estradiol) levels vary during the estrous cycle. Reprod Biol Endocrinol. 9: 34. doi: 10.1186/1477-7827-9-34.

- Franks S. (2005) Polycystic ovary syndrome. Medical Progress. Massachusetts. 333: 853-861.

- Franks S. (2009) Do animal models of polycystic ovary syndrome help to understand its pathogenesis and management? Yes, but their limitations should be recognized. Endocrinology. 150: 3983-3985.

- Fredericks CM, Lundquist LE, Mathur RS, Ashton SH, Landgrebe SC. (1983) Effects of vasoactive intestinal peptide upon ovarian steroids, ovum transport, and fertility in the rabbit. Biol Reprod. 28: 1052-1060.

- Gadducci A, Gargini A, Palla E, Fanucchi A, Genazzani AR. (2005) Polycystic Ovary Syndrome and Gynecological Cancers: is there a link? Gynecol Endocrinol. 20: 200-208.

- Garraza MH, Aguado LI, De Bortoli MA. (2004) *In vitro* effect of neuropeptides on ovary or celiac ganglion affects the release of progesterone from ovaries in the rat. Med Sci Monit. 10: 440-446.

- George FW, Ojeda SR. (1987) Vasoactive intestinal peptide enhances aromatase activity in the neonatal rat ovary before development of primary follicles or responsiveness to follicle-stimulating hormone. Proc Natl Acad Sci. 84: 5803-5807.

- Gilling-Smith C, Willis DS, Beard RW, Franks S. (1994) Hypersecretion of androstenedione by isolated thecal cell from polycystic ovaries. J Clin Endocrinol Metab. 79: 1158-1165.

- Goldfien A, Monroe SE. (2000) Ovarios. En: Greenspan FS, Strewler GJ. (Eds.) Endocrinología básica y clínica. 4a edición. Editorial El Manual Moderno. México, D.F. Cap. 13. pp. 497-554.

- Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. (2011) Polycystic Ovary Syndrome: Etiology, Pathogenesis and Diagnosis. Nat Rev Endocrinol. 7: 219-231.

- Gore-Langton RE, Armstrong DT. (1994) Follicular steroidogenesis and its control. En: Knobil E, Neill JD. (Eds.) The physiology of reproduction. Segunda edición. Raven Press. New York. Vol. 2. Cap. 11. pp. 571-611.

- Gozes I, Tsafriri A. (1986) Detection of vasoactive intestinal peptide-encoding messenger ribonucleic acid in the rat ovaries. Endocrinology. 119: 2606-2610.

- Gräs S, Hedetoft C, Pedersen SH, Fahrenkrug J. (2000) Pituitary adenylate cyclase-activating peptide stimulates acute progesterone production in rat granulosa/lutein cells via two receptor subtypes. Biol Reprod. 63: 206-212.

- Gur EB, Karadeniz M, Turan GA. (2015) Fetal programming of polycystic ovary syndrome. World J Diabetes. 6: 936-942.

- Hall JE. (2009) Neuroendocrine control of the menstrual cycle. En: Strauss JF, Barbieri RL (Eds.) Yen and Jaffe's reproductive endocrinology: physiology, pathophysiology, and clinical management. 6a edición. Elsevier Inc. Philadelphia. Cap. 7. pp. 139-154.

- Heider U, Pedal I, Spanel-Borowski K. (2001) Increase in nerve fibers and loss of mast cells in polycystic and postmenopausal ovaries. Fertil Steril. 75: 1141-1147.

- Hernandez ER, Jimenez JL, Payne DW, Adashi EY. (1988) Adrenergic regulation of ovarian androgen biosynthesis is mediated via β_2-drenergic theca-interstitial cell recognition sites. Endocrinology. 122: 1592-1602.

- Hoyt KL, Schmidt MC. (2004) Polycystic ovary (Stein-Leventhal) syndrome: etiology, complications and treatment. Clin Lab Sci. 17: 155-163.

- Hsueh AJ, Adashi EY, Jones PBC, Welsh TH Jr. (1984) Hormonal regulation of the differentiation of cultured ovarian granulosa cells. Endocr Rev. 5: 76-127.

- Hu J, Zhang Z, Shen WJ, Azhar S. (2010) Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. Nutr Metab (Lond). 7: 47. doi: 10.1186/1743-7075-7-47.

- Hulshof SCJ, Dijkstra G, Van Der Beek EM, Bevers MM, Figueiredo JR, Beckers JF, Van Den Hurk R. (1994) Immunocytochemical localization of vasoactive intestinal peptide and neuropeptide Y in the bovine ovary. Biol Reprod. 50: 553-560.

- Ishihara T, Shigemoto R, Mori K, Takahashi K, Nagata S. (1992) Functional expression and tissue distribution of a novel receptor for vasoactive intestinal polypeptide. Neuron. 8: 811-819.

- Jakubowski L. (2005) Genetic aspects of polycystic ovary syndrome. Endokrynol Pol. 56: 285-293.

- Jamnongjit M, Hammes SR. (2006) Ovarian steroids: the good, the bad, and the signals that raise them. Cell Cycle. 5: 1178-1183.

- Jedel E, Labrie F, Odén A, Holm G, Nilsson L, Janson PO, Lind AK, Ohlsson C, Stener-Victorin E. (2011) Impact of electro-acupuncture and physical exercise on hyperandrogenism and oligo/amenorrhea in women with polycystic ovary syndrome: a randomized controlled trial. Am J Physiol Endocrinol Metab. 300: E37-45.

- Johnson AL, Jean ZLI, Malamed S. (1994) Vasoactive intestinal peptide induced expression of cytochrome p450 cholesterol side chain cleavage and 17α-hidroxilase enzime activity in hen granulosa cells. Biol Reprod. 51: 327-333.

- Kahsar-Miller MD, Conway-Myers BA, Boots LR, Azziz R (2001). Steroidogenic acute regulatory protein (star) in the ovaries of healthy women and those with polycystic ovary syndrome. Am J Obstet Gynecol. 185: 1381-1387.

- Kiener M. (2010) Mecanismos y mediadores químicos involucrados en la ovulación en animales domésticos. Revista FAVE-Ciencias Veterinarias. 9: 39-48.

- Kowalewski MP, Dyson MT, Boos A, Stocco DM. (2010) Vasoactive intestinal peptide (VIP)-mediated expression and function of steroidogenic acute regulatory protein (StAR) in granulosa cells. Mol Cell Endocrinol. 328: 93-103.

- Lara HE, Dissen GA, Leyton V, Paredes A, Fuenzalida H, Fiedler JL, Ojeda SR. (2000) An increased intraovarian synthesis of nerve growth factor and its low affinity receptor is a

principal component of steroid-induced polycystic ovary in the rat. Endocrinology. 141: 1059-1072.

- Lara HE, Ferruz J, Luza S, Bustamante DA, Borges Y, Ojeda SR. (1993) Activation of ovarian sympathetic nerves in polycystic ovary syndrome. Endocrinology. 133: 2690-2695.

- Lawrence Jr. IE, Burden HW. (1980) The origin of the extrinsic adrenergic innervation to the rat ovary. Anat Rec. 196: 51-59.

- Legro RS, Finegood D, Dunaif A. (1998) A fasting Glucose to Insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 83: 2694-2698.

- Legro RS, Kunselman AR, Dodson WC, Dunaif A. (1999) Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab. 84: 165-169.

- Lim SS, Norman RJ, Davies MJ, Moran LJ. (2013) The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. Obes Rev. 14: 95-109.

- Linares R, Hernández D, Morán C, Chavira R, Cárdenas M, Domínguez R, Morales-Ledesma L. (2013) Unilateral or bilateral vagotomy induces ovulation in both ovaries of rats with polycystic ovarian syndrome. Reprod Biol Endocrinol. 11: 68. doi: 10.1186/1477-7827-11-68.

- Liu Y, Kasson BG, Dahl KD, Hsueh AJW. (1987) Vasoactive intestinal peptide stimulates plasminogen activator activity by cultured rat granulosa cells and cumulus-oocyte complexes. Peptides. 8: 29-33.

- Luna SL, Neuman S, Aguilera J, Brown DI, Lara HE. (2012) In vivo β-adrenergic blockade by propranolol prevents isoproterenol-induced polycystic ovary in adult rats. Horm. Metab. Res. 44: 676-681.

- Lutz EM, Sheward WJ, West KM, Morrow JA, Fink G, Harmar AJ. (1993) The VIP2 receptor: molecular characterization of a cDNA encoding a novel receptor for vasoactive intestinal peptide. FEBS Lett. 334: 3-8.

- Manneras L, Cajander S, Holmang A, Seleskovic Z, Lysting T, Lonn M y Stener-Victorin E. (2007) A new rat exhibing both ovarian and metabolic characteristics of polycystic ovary syndrome. Endocrinology. 148: 3781-3791.

- March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. (2010) The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 25: 544-551.

- Mayerhofer A, Dissen GA, Costa ME, Ojeda SR. (1997) A role for neurotransmitters in early follicular development: induction of functional follicle-stimulating hormone receptors in newly formed follicles of the rat ovary. Endocrinology. 138: 3320-3329.

- Mehrabian F, Khani B, Kelishadi R, Ghanbari E. (2011) The prevalence of polycystic ovary syndrome in Iranian women based on different diagnostic criteria. Endokrynol Pol. 62: 238-242.

- Merino P, Schulin-Zeuthen C, Codner E. (2009) Current diagnosis of polycystic ovary syndrome: expanding the phenotype but generating new questions. Rev Med Chil. 137: 1071-1080.

- Michelmore KF, Balen AH, Dunger DB, Vessey MP. (1999) Polycystic ovaries and associated clinical and biochemical features in young women. Clin Endocrinol (Oxf). 51: 779-786.

- Miller WL, Auchus RJ. (2011) The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocrine Reviews. 32: 81-151.

- Morales-Ledesma L, Linares R, Rosas G, Morán C, Chavira R, Cárdenas M, Domínguez R. (2010) Unilateral sectioning of the superior ovarian nerve of rats with polycystic ovarian syndrome restores ovulation in the innervated ovary. Reprod Biol Endocrinol. 8: 99. doi: 10.1186/1477-7827-8-99.

- Morán, C., Franco, A., Morán, J.L., Handal, A., Morales, L., Domínguez, R.: Neural activity between ovaries and the prevertebral celiac-superior mesenteric ganglia varies during the estrous cycle of the rat. Endocrine. 26, 147-152 (2005)

- Navaratnarajah R, Pillay OC, Hardiman P. (2008) Polycystic ovary syndrome and endometrial cancer. Semin Reprod Med. 26: 62-71.

- Norman AW, Litwack G. (1997) Hormones. Segunda edición. Academic Press. San Diego, USA. Cap. 2. pp. 49-86.

- Olivares RJA, Arellano PA. (2008) Bases moleculares de las acciones de la insulina. REB. 27: 9-18.

- Padmanabhan V, Manikkam M, Recabarren S, Foster D. (2006) Prenatal testosterone excess programs reproductive and metabolic dysfunction in the female. Mol Cell Endocrinol. 246: 165-174.

- Pala L, Barbaro V, Dicembrini I, Rotella CM. (2014) The therapy of insulin resistance in other diseases besides type 2 diabetes. Eat Weight Disord. 19: 275-283.

- Palomba S, Santagni S, Falbo A, La Sala GB. (2015) Complications and challenges associated with polycystic ovary syndrome: current perspectives. Int J Womens Health. 7: 745-763.

- Paredes A, Gálvez A, Leyton V, Aravena G, Fiedler JL, Bustamante D, Lara HE. (1998) Stress promotes development of ovarian cysts in rats the possible role of sympathetic nerve activation. 8: 309-315.

- Parra C, Fiedler JL, Luna SL, Greiner M, Padmanabhan V, Lara HE. (2007) Participation of vasoactive intestinal polypeptide in ovarian steroids production during the rat estrous cycle and in the development of estradiol valerate-induced polycystic ovary. Reproduction. 133: 147-154.

- Ramírez HDA. (2011) ¿La Participación del Nervio Ovárico Superior en la Regulación de la Ovulación y la Esteroidogénesis Depende del Ciclo Circádico? La Rata Adulta como Modelo de Estudio. Tesis de Maestría. UNAM, FES Zaragoza.

- Ramírez SMI. (2014) Efecto del bloqueo de los receptores del péptido intestinal vasoactivo en el ovario de la rata con síndrome del ovario poliquístico, sobre la regulación de la secreción de progesterona y estradiol. Tesis de Licenciatura. UNAM, FES Zaragoza.

- Ratner A, Weiss GK, Sanborn CR. (1980) Stimulation by β_2-adrenergic receptors of the production of cyclic AMP and progesterone in rat ovarian tissue. J Endocrinol. 87: 123-129.

- Rebar R, Judd HL, Yen SS, Rakoff J, Vandenberg G, Naftolin F. (1976) Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. J Clin Invest. 57: 1320-1329.

- Romero C, Paredes A, Dissen GA, Ojeda SR. (2002) Nerve growth factor induces the expression of functional fsh receptors in newly formed follicles of the rat ovary. Endocrinology. 143: 1485-1494.

- Rosa-E-Silva A, Guimaraes MA, Padmanabhan V, Lara HE. (2003) Prepubertal administration of valerate disrupts cyclicity and leads to cystic ovarian morphology during adult life in the rat: role of sympathetic innervation. Endocrinology. 144: 4289-4297.

- Rosas GG. (2006) Efecto de la inducción del síndrome del ovario poliquístico en la rata infantil, sobre la dinámica del crecimiento folicular y la secreción de hormonas esteroides. Tesis de Licenciatura. UNAM, FES Zaragoza.

- Rosas GG. (2011) Participación del péptido intestinal vasoactivo en la regulación de la ovulación y la secreción hormonal. La rata con síndrome del ovario poliquístico como modelo de estudio. Tesis de Maestría. UNAM, FES Zaragoza.

- Rosenfield RL, Barnes RB, Carac JF, Lucky AW. (1990) Dysregulation of cytochrome p450c17 alpha as the cause of polycystic ovary syndrome. Fertil Steril. 53: 785-791.

- Rosenfield RL. (1999) Ovarian and adrenal function in polycystic ovary syndrome. Endocrinol Metab Clin N Am. 28: 265-268.

- Ruíz KV, Chavira R, Domínguez R, Morales L. (2008) Efecto de la denervación catecolaminérgica del ovario realizada desde la etapa infantil, sobre el desarrollo del síndrome del ovario poliquístico en la rata. LI Congreso Nacional de Ciencias Fisiológicas. Cd. de Mérida, Yucatán.

- Said SI, Rosenberg RN. (1976) Vasoactive intestinal polypeptide: abundant immunoreactivity in neural cell lines and normal nervous tissue. Science. 192: 907-908.

- Schmidt G, Jörgensen J, Kannisto P, Liedberg F, Ottesen B, Owman Ch. (1990) Vasoactive intestinal polypeptide in the PMSG-primed immature rat ovary and its effects on ovulation in the isolated rat ovary perfused in vitro. J Reprod Fert. 90: 465-472.

- Schultea TD, Dees WL., Ojeda SR. (1992) Postnatal development of sympathetic and sensory innervation of the rhesus monkey ovary. Biol. Reprod. 47: 760-767.

- Selvaraj N, Dantes A, Amsterdam A. (2000) Establishment and characterization of steroidogenic granulosa cells expressing β_2-adrenergic receptor: regulation of adrenodoxin and steroidogenic acute regulatory protein by adrenergic agents. Mol Cell Endocrinol. 168: 53-63.

- Semenova II. (1969) Adrenergic innervation of ovaries in Stein-Leventhal syndrome. Vestn Akad Med Nauk SSSR. 24: 58-62.

- Shi D, Vine DF. (2012) Animal models of polycystic ovary syndrome: a focused review of rodent models in relationship to clinical phenotypes and cardiometabolic risk. Fertil Steril. 98: 185-193.

- Sir-Petermann T, Codner E, Pérez V, Echiburú B, Maliqueo M, Ladrón de Guevara A, Preisler J, Crisosto N, Sánchez F, Cassorla F, Bhasin S. (2009). Metabolic and reproductive features before and during puberty in daughters of women with polycystic ovary syndrome. J Clin Endocrinol Metab. 94: 1923-1930.

- Spicer LJ. (1986) Catecholaminergic regulation of ovarian function in mammals: current concepts. Life Sci. 39: 1701-1711.

- Stener-Victorin E, Jedel E, Janson PO, Sverrisdottir YB. (2009) Low-frequency electroacupuncture and physical exercise decrease high muscle sympathetic nerve activity in polycystic ovary syndrome. Am J Physiol Regul Integr Comp Physiol. 297: R387-395.

- Stener-Victorin E, Lundeberg T, Waldenstrom U, Manni L, Aloe L, Gunnarsson S, Olof P. (2000b) Effects of electro-acupuncture on nerve growth factor and ovarian morphology in rats with experimentally induced polycystic ovaries. Biol Reprod. 63: 1497-1503.

- Stener-Victorin E, Waldenström U, Tägnfors U, Lundeberg T, Lindstedt G, Janson PO. (2000a) Effects of electro-acupuncture on anovulation in women with polycystic ovary syndrome. Acta Obstet Gynecol Scand. 79: 180-188.

- Strauss JF, Williams CJ. (2009) The Ovarian Life Cycle. En: Strauss JF, Barbieri RL (Eds.) Yen and Jaffe's Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management. 6th Edition. Elsevier Inc. Philadelphia. Cap. 8, pp. 155-190.

- Strauss JF. (2009) The synthesis and metabolism of steroid hormones. En: Strauss JF, Barbieri RL (Eds.) Yen and Jaffe's reproductive endocrinology: physiology, pathophysiology, and clinical management. 6th Edition. Elsevier Inc. Philadelphia. Cap. 4. pp. 79-104.

- Tajima K, Orisaka M, Mori T, Kotsuji F. (2007) Ovarian theca cells in follicular function. Reprod Biomed Online. 15: 591-609.

- Takayama K, Fukaya T, Sasano H, Funayama Y, Suzuki T, Takaya R, Wada Y, Yajima A. (1996) Immunohistochemical study of steroidogenesis and cell proliferation in polycystic ovarian syndrome. Hum Reprod. 11: 1387-1392.

- Talbot JA, Bicknell EJ, Rajkhowa M, Krook A, O'Rahilly S, Clayton RN. (1996) Molecular scanning of the insulin receptor gene in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 81: 1979-1983.

- Taylor AE, McCourt B, Martin KA, Anderson EJ, Adams JM, Schoenfeld D, Hall JE. (1997) Determinants of abnormal gonadotropin secretion in clinically defined women with polycystic ovary syndrome. J Clin Endocrinol Metab. 82: 2248-2256.

- Taylor AE. (1998) Understanding the underlying metabolic abnormalities of polycystic ovary syndrome and their implications. Am J Obstet Gynecol. 179: 94-100.

- Tilly JL, Johnson AL. (1989) Regulation of androstenedione production by adenosine 3´, 5´-monophosphate and phorbol myristate acetate in ovarian thecal cell of the domestic hen. Endocrinology. 125: 1691-1699.

- Törnell J, Carlsson B, Hillensjö T. (1988) Vasoactive intestinal peptide stimulates oocyte maturation, steroidogenesis, and cyclic adenosine 3',5'-monophosphate production in isolated preovulatory rat follicles. Biol Reprod. 39: 213-220.

- Trzeciak WH, Ahmed CE, Simpson ER, Ojeda SR. (1986) Vasoactive intestinal peptide induces the synthesis of the cholesterol side-chan cleavage enzyme complex in cultured rat ovarian granulosa cells. Proc Natl Acad Sci. 83: 7490-7494.

- Wachs DS, Coffler MS, Malcom PJ, Shimasaki S, Chang RJ. (2008) Increased androgen response to follicle-stimulating hormone administration in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 93: 1827-1833.

- Waldstreicher J, Santoro NF, Hall JE, Filicori M, Crowley WF Jr. (1988). Hyperfunction of the hypothalamic–pituitary axis in women with polycystic ovarian disease: indirect evidence for partial gonadotroph desensitization. J Clin Endocrinol Metab. 66: 165-172.

- Walters KA, Allan CM, Handelsman DJ. (2012) Rodent models for human polycystic ovary syndrome. Biol Reprod. 86: 149, 1-12. doi: 10.1095/biolreprod.111.097808.

- Wang S, Liu G, Ahmad S, Liu W, Riaz H, Jiang X. (2012) Vasoactive intestinal peptide suppresses ovarian granulosa cell apoptosis in vitro by up-regulating Bcl-2 gene expression. Anim Reprod Sci. 132: 201-206.

- Wasilewska-Dziubińska E, Borowiec M, Chmielowska M, Woliñska-Witort E, Baranowska B. (2002) Alfa 1 adrenergic potentiation of progesterone accumulation stimulated by vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) in cultured rat granulosa cells. Neuro Endocrinol. Lett. 23: 141-148.

- Weiss GK, Dail WG, Ratner A. (1982) Evidence for direct neural control of ovarian steroidogenesis in rats. J. Reprod. Fertil. 65: 507-511.

- Wu XY, Li ZL, Wu CY, Liu YM, Lin H, Wang SH, Xiao WF. (2010) Endocrine traits of polycystic ovary syndrome in prenatally androgenized female Sprague-Dawley rats. Endocr J. 57: 201-209.

- Yeh J, Adashi EY. (2001) El ciclo ovárico. En: Yen SSC, Jaffe RB, Barbieri RL. (Eds.) Endocrinología de la reproducción. 4a edición. Editorial Médica Panamericana. Buenos Aires. Cap. 6. pp. 164-202.

- Zangeneh FZ, Abdollahi A, Tavassoli P, Naghizadeh MM. (2011) The effect of cold stress on polycystic ovary syndrome in rat: before and during modeling. Arch Gynecol Obstet. 284: 651-657.

- Zawadzki JK, Dunaif A. (1992) Diagnostic criteria for polycystic syndrome: towards a rational approach. En: Dunaif A, Givens JR, Haseltine FP, Merriam GR. (Eds.) Polycystic Ovary Syndrome. Boston. Blackwell Scientific Publications. pp. 377.

- Zhang LH, Rodriguez H, Ohno S, Miller WL. (1995) Serine phosphorylation of human P450c17 increases 17, 20-lyase activity: implications for adrenarche and the polycystic ovary syndrome. Proc Natl Acad Sci U S A. 92: 10619-10623.