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SECRETION FROM NEUROPEPTIDE-TREATED SPLENOCYTES MODIFIES OVARIAN STEROIDOGENESIS

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Abstract There are evidences for modulation of immune function by the sympathetic nervous system and its principal neurotransmitter norepinephrine (NE) through superior ovarian nerve (SON)-coeliac ganglion-noradrenergic postganglionic innervation of the spleen. Seven days after SON transection at 53 days of age, the rat splenocytes were isolated and then cultured for 48 h. These culture media, used to stimulate ovaries from 60-day-old intact rats (neither SON-transected nor sham-operated) at diestrus 2 stage, in *in vitro* incubations, showed a decrease in progesterone release, an increase in estradiol release and no change in androstenedione release in relation to splenocyte culture media from control (sham-operated) rats. When esplenocytes from SON transected (SON-t) rats were treated with vasoactive intestinal peptide (VIP) or neuropeptide Y (NPY), both at 10^{-6}M for 24 h, their secretions increased the progesterone release while decreasing the estradiol release from the intact ovaries, compared with the secretions of untreated splenocytes from SON-t rats. Although the secretions of splenocytes treated with VIP decrease the androstenedione release from the ovaries, the treatment with NPY produced no change in hormone release. In the present paper the ovarian steroidogenic response, which was modified by the effects of an in vivo SON transection on spleen cells, was reverted by an *in vitro* system in which the splenocytes were treated with VIP or NPY. This could indicate that the spleen of SON-t rats does not receive those neuropeptides by neural route however, when they are added to splenocyte culture *in vitro*, the cell secretions revert the profile of steroid hormones released from the intact ovary. We also present functional evidence for modulation of the immune function by sympathetic nervous system and neurotransmitters other than NE.

Key words: splenocytes, vasoactive intestinal peptide, neuropeptide Y, ovarian steroidogenesis.

Resumen ***Secreciones de esplenocitos tratados con neuropéptidos modifican la esteroidogénesis ovárica.*** Algunas evidencias indican que la función inmune está regulada por el sistema nervioso simpático y su neurotransmisor principal, norepinefrina (NE), a través del sistema nervio ovárico superior (NOS)-ganglio celíaco-inervación posganglionar noradrenérgica del bazo. En el presente trabajo, se seccionó el NOS a ratas de 53 días de edad y se sacrificaron los animales siete días más tarde (60 días de edad). Se aislaron los esplenocitos del bazo y se cultivaron por 48 hs. Se trabajó paralelamente con ratas controles (con operación simulada). El medio de cultivo se usó para estimular ovarios de ratas intactas de 60 días en diestro 2 (sin el NOS seccionado o con operación simulada) en incubaciones *in vitro.* Se observó disminución de la progesterona y aumento del estradiol liberados por el ovario, en relación al efecto producido por el medio de cultivo de esplenocitos de ratas controles. Cuando los esplenocitos de ratas con el NOS seccionado (NOS-s) fueron tratados con péptido intestinal vasoactivo (VIP) o con neuropéptido Y (NPY), 10^{-6}M durante 24 h, sus secreciones incrementaron la liberación de progesterona, mientras que disminuyeron la de estradiol, desde ovarios intactos, comparado con las secreciones de los esplenocitos no tratados de ratas NOS-s. Las secreciones de esplenocitos tratados con VIP disminuyeron la liberación de androstenodiona desde ovarios intactos, mientras que con NPY no se observaron cambios significativos. La respuesta esteroidogénica ovárica, que fue modificada por los efectos de la sección del NOS sobre las células del bazo, fue revertida cuando los esplenocitos de ratas NOS-s fueron tratados *in vitro* con VIP or NPY. Esto sugiere que la sección del NOS disminuye la llegada de VIP y NPY al bazo. Cuando se adicionan dichos neuropéptidos a los esplenocitos en cultivo, las secreciones de estas células revierten el perfil de las hormonas liberadas por ovarios intactos. En este trabajo también se muestra evidencia de la modulación de la función inmune por el sistema nervioso simpático y por neurotransmisores distintos a NE.

Palabras clave: esplenocitos, péptido intestinal, vasoactivo, neuropéptido Y, esteroidogénesis ovárica

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Communication between the neuroimmune and endocrine systems and a reciprocal flow of information are suggested by several observations[1]. It is known that sympathetic noradrenergic (NA) nerve fibers and sensory neurons inervate immune organs such as the spleen, the thymus, the lymph nodes and gut-associated lymphoid tissues[2-4]. On the other hand, the ability of various hormones to affect immune functions *in vivo* and *in vitro* has long been known. An elevation of adrenal glucocorticoids during stress has been considered to be responsible for stress-induced immunosuppression[5]. LH, FSH, PRL, GH, TSH, among others, have been shown to modulate the immune response[6, 7].

The noradrenergic postganglionic innervation of rat spleen originates mainly in the superior mesenteric/ coeliac ganglion and the nerve fibers enter the spleen at the hilar region with the vasculature[8, 9]. Electron microscopic immunocytochemical studies of tyrosine hydroxylase nerve fibers have shown direct contacts of nerve terminals with lymphocytes and macrophages in the spleen[10]. The endings of these nerves contain norepinephrine (NE), and, among other neuropeptides, vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY)[11, 12]. The presence of NE, VIP and NPY receptors in rat splenic lymphocytes has been demonstrated[12-14]. The reduction in splenic NA innervation is associated with degeneration of NA nerve fibers and ultimately, with loss of their cell bodies in the superior mesenteric/coeliac ganglionic complex[15].

On the other hand, it is known that the superior ovarian nerve (SON), which innervates the ovary, has its preganglionic cell bodies in the spinal cord[16]. These exit at thoraco spinal cord at levels T10 and T11 and synapse in neurons of coeliac and suprarrenal ganglia. It has been demonstrated that the SON contains mainly afferent and efferent noradrenergic (NA) fibers and also vipiergic fibers from the coeliac ganglion[17-19]. SON transection in adult rats affects splenocyte secretions, which participate in the ovarian steroidogenic response, particularly in progesterone release, which secretions might be controlled by adrenergic influences since the number of splenocyte β-adrenergic receptors changes through SON-coeliac ganglion-noradrenergic postganglionic innervation of the spleen[20]. There is almost no information about the effect of SON transection on the spleen cells, which, like the ovary, are also innervated extensively by afferent and efferent noradrenergic sympathetic nerve fibers from the coeliac ganglion[16, 17].

The aim of the present work was to study if the effects of an *in vivo* SON transection on the spleen cells, determined by the effect of the secretions of those cells on the steroidogenic response of intact ovaries, could be reverted by an *in vitro* system in which the splenocytes were treated with VIP or NPY, as we have previously done with NE[20]. Culture media of splenocytes from SON transected (SON-t) rats and of those splenocytes treated with VIP or NPY were used to stimulate intact ovaries.

Materials and Methods

Chemicals. Liquid culture medium, penicillin G, streptomycin, crude trypsin, soybean trypsin inhibitor, VIP and NPY were purchased from Sigma Chemical Co (St. Louis, MO, USA). Progesterone, estradiol, androstenedione were provided by New England Nuclear (Boston, MA, USA). All the other chemicals were of reagent grade and were purchased from Merck Laboratory, Buenos Aires, Argentina.

Animals and experimental model. Adult female Holtzman rats were used. They were housed in a controlled environment (22-24 ºC, 12 h light- 12 h dark). Water and food were available *ad libitum*. Two groups of cyclic adult rats were used, one SON-transected (SON-t) and the other one sham-operated (control) at 53 days of age. Seven days after the SON transection was performed all rats were killed and the splenocytes were isolated. The splenocytes from SON-t and control rats were preincubated for 24 h, then, the medium was removed and the cells were cultured for an additional 48 h. On the other hand, splenocytes from SON-t rats were cultured in presence of VIP in one case and NPY in another, both at 10^{-6} M, for 24 h. After that, the medium was replaced by fresh medium and the splenocytes were cultured for an additional 24 h. Fluids were collected and used to stimulate ovaries from 60-day-old intact rats (neither SON-t nor sham-operated) rats in diestrus 2 stage, in *in vitro* incubation to measure the steroidogenic response (Fig.1). Considering the influence of SON transection on both the ovary and the spleen, via the coeliac ganglion, in all the experiments carried out, the effect of the culture medium from splenocytes was studied on ovaries from intact rats.

Transection of the superior ovarian nerve. Rats were lightly anaesthetized with ether and the ovaries were exposed through bilateral dorsal incisions. The SON transection was achieved as described previously[20]. In brief, the suspensory ligament enclosing the SON was lifted with fine forceps, the nerve was cut with small scissors, the ovaries were returned to the abdominal cavity, and the wounds were sutured. The suspensory ligament is clearly visible and the SON travels along it, thus, we assumed that transection of the suspensory ligament results in transection of the SON.

Splenocyte cultures. In brief, rats were killed by decapitation, their spleens were aseptically removed, washed in saline solution and pressed through a sterile nylon screen (200 mm mesh) to obtain individual splenocytes. The cells were collected by centrifugation (600 g, 5 min,) resuspended in serum-free RPMI medium and treated with 0.83% NH_4Cl: 2.6% TRIS (9:1, v/v) pH 7.2, to remove the red blood cells. Cells (1 x 10^6 viable cells/ml of medium) were cultured in 8 x 75 mm glass test tubes at 37 ºC in 95% air: 5% CO_2 atmosphere. The medium was supplemented with 10% fetal bovine serum and antibiotics (50 mg/ml streptomycin and 50 units/ml penicillin).

Ovary incubation. After killing the intact rats, the ovaries were immediately removed, dissected, weighed and preincubated in 1 ml of fresh culture medium at 37 ºC, in 95% O_2: 5% CO_2 mixture. After 30 min, the medium was discarded and 1 ml of splenocyte culture medium was added. Incubation was continued for 3 h and then the medium was removed and stored at -70 ºC for subsequent measurement of steroids.

Steroid assays. The progesterone, estradiol and androstenedione contents of culture media were determined by specific RIAs using antisera as described previously[21]. The assay sensitivity was less than 5 ng/ml for progesterone, 12 fmole/ tube for estradiol and 0.02 ng/ml for androstenedione. The inter-

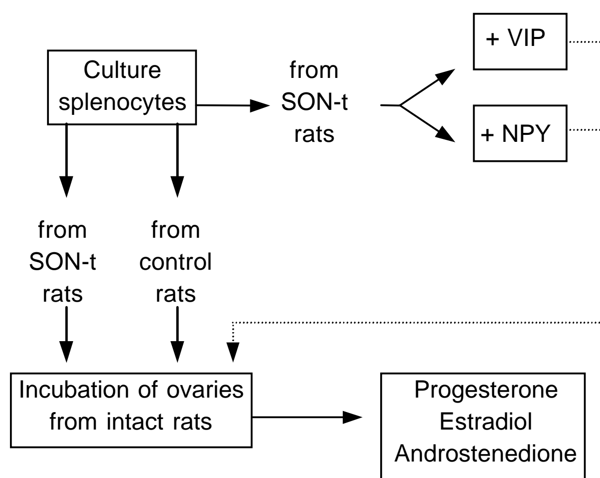


Fig. 1.– Diagram of the experimental design.

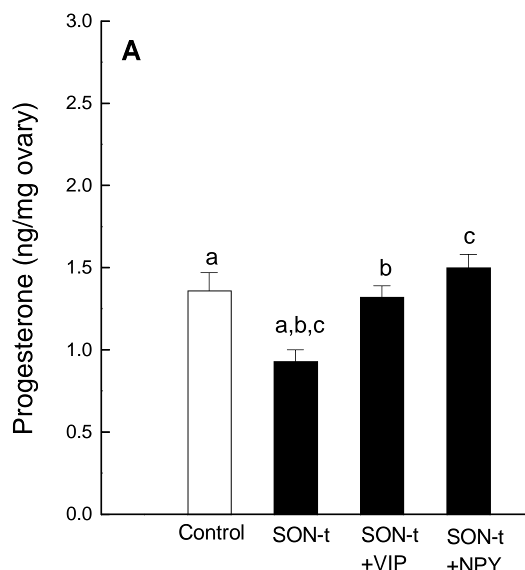
and intraassay coefficients of variation for all the assays were less than 10.0%.

Statistical analyses. Results are expressed as mean ± SEM. Significant differences among means were considered at a level of P< 0.05 and tested by Student's *t*-test.

Results

The effect of secretions from splenocytes treated with neuropeptides on the ovarian steroidogenesis is shown in the figures. The results were expressed in all cases as ng or pg / mg ovary or / mg protein. Fig. 2 shows the progesterone release from ovaries of control and SON-t rats incubated with culture medium of splenocytes. When culture medium of splenocytes from SON-t rats was used, the progesterone release from ovaries decreased significantly in relation to that observed when the splenocyte culture medium came from control rats, p< 0.001. When splenocytes from SON-t rats were treated with VIP or NPY, their secretions increased the progesterone release from the ovaries (p< 0.001), compared with the secretions from SON-t rats untreated splenocytes. The values obtained in presence of neuropeptides reached the progesterone levels released by the ovaries incubated with culture medium from splenocytes of control rats (p< 0.001).

As shown in Fig. 3, the estradiol release from ovaries incubated with culture medium of SON-t rat splenocytes without neuropeptide treatment increased markedly in relation to that obtained with culture medium of splenocytes from control rats, p< 0.001. However, when culture medium of SON-t rat splenocytes treated with VIP or NPY was used, a significant decrease (p< 0.001) in estradiol release from ovaries was observed, compared with that from ovaries incubated with secretions of SON-t rat untreated splenocytes.



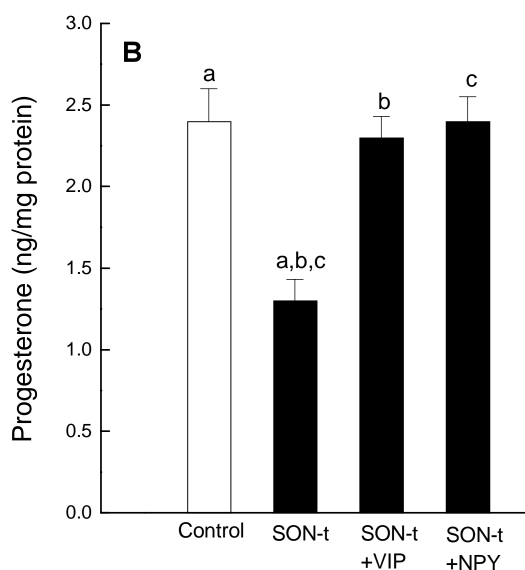
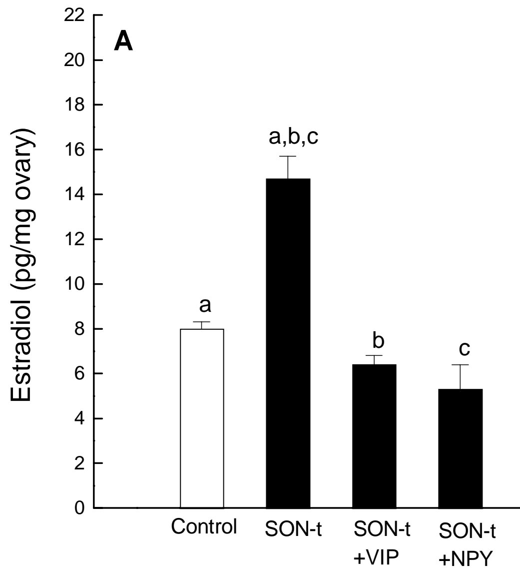
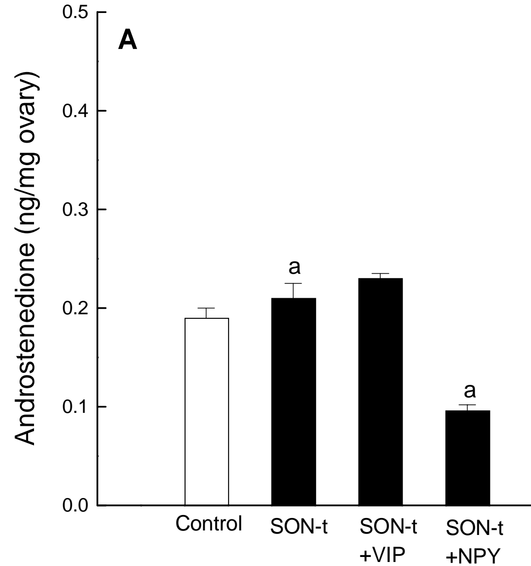
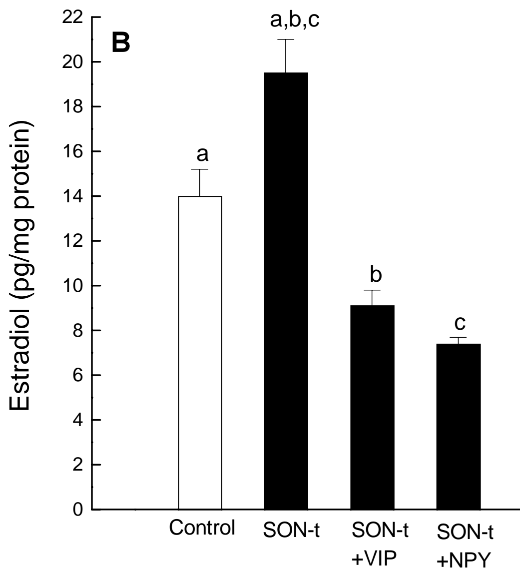


Fig. 2.– «In vitro» progesterone release from ovaries of 60-day-old intact rats stimulated with culture medium of splenocytes from control (open bars) and SON-t (solid bars) rats. Secretions from SON-t rat splenocytes incubated 24 h with and without VIP or NPY, both at 10^{-6}M, and then cultured in fresh medium alone for another 24 h were used. **A**) Progesterone ng / mg ovary. **B**) Progesterone ng / mg protein. Values are expressed as the mean ± SEM of triplicate determinations of 2 different experiments, using 4 rats per group per experiment (total 16 ovaries per group). Bars sharing a same superscript letter indicate significant differences at ^{a,b,c} p<0.001 as determined by Student's *t*- test.

When culture medium of splenocytes from SON-t rats was used, the androstenedione release from ovaries did not change in relation to that observed when the splenocyte culture medium derived from control rats.







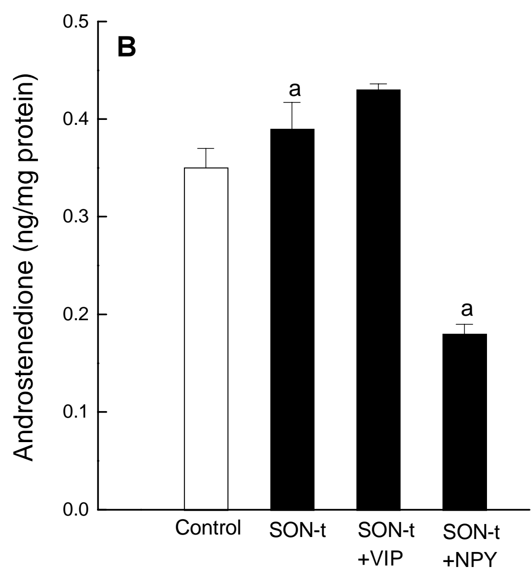


Fig. 3.- «In vitro» estradiol release from ovaries of 60-day-old intact rats stimulated with culture medium of splenocytes from control (open bars) and SON-t (solid bars) rats. Treatment of splenocytes with VIP or NPY as well as the number of determinations and experiments were done as indicated in legend of Fig. 2. **A**) Estradiol pg / mg ovary. **B**) Estradiol pg / mg protein. Values are expressed as the mean ± SEM. Bars sharing a same superscript letter indicate significant differences at [a,b,c] $p<0.001$ as determined by Student's *t*- test.

Fig. 4.- «In vitro» androstenedione release from ovaries of 60-day-old intact rats stimulated with culture medium of splenocytes from control (open bars) and SON-t (solid bars) rats. Treatment of splenocytes with VIP or NPY as well as the number of determinations and experiments were done as indicated in legend of Fig. 2. **A**) Androstenedione ng / mg ovary. **B**) Androstenedione ng / mg protein. Values are expressed as the mean ± SEM. Bars sharing a same superscript letter indicate significant differences at [a] $p<0.001$ as determined by Student's *t*- test.

Similar results were obtained when splenocytes from SON-t rats were previously treated with VIP. The secretions of splenocytes treated with NPY decreased ($p< 0.001$) the androstenedione release from the ovaries (Fig. 4).

Discussion

The ovary and spleen are innervated extensively by afferent and efferent noradrenergic sympathetic nerve fibers from the coeliac ganglion[16, 17, 22, 23]. The endings of

those nerves contain NE, VIP and NPY[11, 12], among other neurotransmitters. In particular, the coeliac ganglion is connected through the superior ovarian nerve to the ovary [16, 17, 24]. We have previously shown that the effects on the splenocyte β-adrenergic receptors number after an in vivo SON transection were simulated by an in vitro system modulating β-adrenergic receptors number by treatment of splenocytes from SON-t rats with and without NE. Additionaly, we have observed that the change in the ß-adrenergic receptors number was associated to a change in the secretion of those splenocytes since ovaries from intact rats incubated with those secretions modify their steroidogenic response. It was suggested that the activity of splenocytes could be controlled by adrenergic influences[20]. In the present investigation we showed that the effects of an *in vivo* SON transection on the splenocyte secretions could be reverted by an *in vitro* system in which the splenocytes were treated with VIP or NPY, as we have previously done with NE[20]. We showed that the secretions of the cultured splenocytes from SON-t rats produced a decrease of progesterone, an increase of estradiol and no change in androstenedione release from intact ovaries. It has been observed that circulating levels of IL-1 are elevated during luteal phase of normally cycling women[25]. Inversely, low concentrations of progesterone appear to upregulate macrofage IL-1 gene expression, while higher concentrations of progesterone significantly inhibit IL-1 activity[26]. In our case, low concentrations of progesterone were released from the ovaries when they were incubated with secretions of the cultured splenocytes from SON-t rats, suggesting that those secretions are not the same as those of splenocytes from control rats. This fact could be in agreement with the eventual participation of IL-1 in the process. In an *in vivo* situation, there is no possibility that high levels of progesterone affect the splenocyte secretions, since the circulating progesterone levels did not change with the SON transection (data not shown). We also show that splenocyte secretions of SON-t rats stimulate estrogen release from the ovaries, indicating that the SON transection modifies the splenocyte secretions. It has been demonstrated in granulosa cells that the steroidogenic activity of a factor obtained from the splenocytes of normal animals could not be attributed to a FSH-like molecule. Those authors suggest that that factor is different from FSH because it does not contain FSH immunoreactivity nor does it contain FSH receptor-binding activity[27].

It is known that rat splenic lymphocytes possess VIP and NPY receptors[12-14]. In this paper we show evidence that splenocyte secretions, which participate in the ovarian steroidogenic response, might be controlled by VIP and NPY. We observed that culture medium of SON-t rats, containing the secretions of VIP treated or NPY treated splenocytes increased the progesterone release from the ovaries, while the release of estradiol decreased, compared with the secretions of untreated splenocytes. On the other hand, the secretions of NPY treated splenocytes decreased the androstenedione release from the ovaries, but no change was observed when splenocytes were treated with VIP. Probably, the decrease in estradiol release, when ovaries were stimulated with secretions of splenocytes treated with NPY, could be associated with the decrease in androstenedione release in the same conditions, considering that androstenedione is a natural precursor of estradiol. Recently, it has been shown that NPY greatly enhanced IL-4 production and inhibited that of interferon-gamma (IFN-gamma) from mouse helper T cell subsets. However, VIP had no effects on IFN-gamma production, while it inhibited IL-4 production slightly but consistently[28]. It has been shown that the addition of nerve growth factor (NGF) to unstimulated purified T cells induces the synthesis of NPY[29]. Thus, these neuropeptides modify the activity of the immune cells. However, the effect of the immune system on ovarian steroidogenic activity is still not clear.

Considering our previous report[20] and the results presented in this study, we can speculate that the SON transection could modify the coeliac ganglion, in a retrograde way, affecting the release of VIP and NPY through its sympathetic fibers to the spleen. This fact could modify the splenocyte activity, probably by releasing specific secretions which, as we have shown, influence the steroidogenic response of intact ovaries. That steroidogenic response was reverted when ovaries were incubated with culture medium of SON-t rats splenocytes treated with VIP or NPY. The factor(s) secreted by splenocytes might provide a link between intraovarian spleen cells and the regulation of the ovarian function. It is known that lymphocytes release specific cytokines which modulate granulosa cell steroidogenesis, typically in an inhibitory[30] and stimulatory fashion[31], but we cannot affirm that the ovarian steroidogenic-modulating factor(s) produced by the splenocytes treated with VIP or NPY is (are) a cytokine.

The relevance of the factor(s) secreted by the splenocytes to the ovarian physiology remains a matter of study but, it is possible that that secretion, either in the spleen or in the circulation, could affect the ovarian function through an endocrine mechanism.

On the other hand, knowing that immune signals influence the hypothalamic-pituitary axis[32] it is possible that the factor(s) secreted by the splenocytes in the intact animal could be released into the general circulation to act on hypothalamic-pituitary sites and subsequently originate modifications in the ovarian function.

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References

1. Gaillard RC. Neuroendocrine-immune system interactions. The immune-hypothalamo-pituitary-adrenal axis. *Trends Endocrinol Metab* 1994; 5: 303-9.
2. Madden KS, Sanders VM, Felten DL. Catecholamine influences and sympathetic neural modulation of inmune responsiveness. *Annu Rev Pharmacol Toxicol* 1995; 35: 417-48.
3. Hori T, Katafuchi T, Take S, Shimizu N, Niijima A. The autonomic nervous system as a communication channel between the brain and the immune system. *Neuroimmunomodulation* 1995; 2: 203-15.
4. Felten SY, Olschowka JA. Noradrenergic sympathetic innervation of the spleen: II. Tyrosine hydroxylase (TH)-positive nerve terminals form synaptic-like contacts on lymphocytes in the splenic white pulp. *J Neurosci Res* 1987; 18: 37-48.
5. Wilcknes T. Glucocorticoids and immune function: physiologycal relevance and pathogenic potential of hormonal dysfunction. *Tips* 1995; 16: 193-7.
6. Rouabhia M, Chakir J, Deschaux P. Interaction between the immune and endocrine systems: immunomodulatory effects of luteinizing hormone. *Prog Neuroendocrinol Immunol* 1991; 4: 86-91.
7. Weigent DA, Blalock JE. Associations between the neuroendocrine and inmune systems. *J Leukoc Biol* 1995; 57: 137-50.
8. Bellinger DL, Felten SY, Lorton D, Felten DL. Origin of noradrenergic innervation of the spleen in rats. *Brain Behav Immunol* 1989; 3: 291-311.
9. Nance DM, Burns J. Innervation of the spleen in the rat: evidence for absence of afferent innervation. *Brain Behav Immunol* 1989; 3: 218-30.
10. Meltzer JC, Grimm PC, Greemberg AH, Nance DM. Enhanced immunohistochemical detection of autonomic nerve fibers, cytokines and inducible nitric oxide syntethase by light and fluorescent microscopy in rat spleen. *J Histochem Cytochem* 1997; 45: 599-610.
11. Romano TA, Felten SY, Felten DL, Olschowka JA. Neuropeptide-Y innervation of the rat spleen: Another potential immunomodulatory neuropeptide. *Brain Behav Immun* 1991; 5: 116-31.
12. Goetzl EJ, Pankhaniya RR, Gaufo GO, MuY, Xia M, Sreedharan SP. Selectivity of effects of vasoactive intestinal peptide on macrophages and lymphocytes in compartmental immune responses. *Neuroimmunomodulation* 1998; 840: 540-50.
13. Petitto JM, Huang Z, McCarthy DB. Molecular cloning of NPY-Y1 receptor cDNA from rat splenic lymphocytes: evidence of low levels of mRNA expression and [^{125}I]NPY binding sites. *J Neuroimmunol* 1994; 54: 81-6.
14. Schwarz H, Villinger PM, von Kempis J, Lotz M. Neuropeptide Y is an inducible gene in the human immune system. *J Neuroimmunol* 1994; 51: 53-61.
15. Santer RM, Partanen M, Hervonen A. Glyoxylic acid fluorescence and ultraestructural studies of neurones in the coeliac-superior mesenteric ganglion of the aged rat. *Cell Tissue Res* 1980; 212: 475-85.
16. Dissen GA, Dees WL, Ojeda SR. Neural and neurotropic control of ovarian development. In: Adashi EY, Leung PCK (eds): The Ovary. New York: Raven Press, 1993, p 1-19
17. Lawrence IE, Burden HW. The origin of the extrinsic adrenergic innervation to the rat ovary. *Anat Rec* 1980; 196: 51-9.
18. Burden HW, Leonard M, Smith CP, Lawrence IE Jr. The sensory innervation of the ovary a horseradish peroxidase study in the rat. *Anat Rec* 1982; 207: 623-7.
19. Jarret WA, Price GT, Lynn VJ, Burden HW. NADPH-diaphorase positive neurons innervating the rat ovary. *Neurosci Lett* 1994; 177: 45-9.
20. Forneris M, Oliveros L, Aguado L. Effect of secretion of splenocytes after superior ovarian nerve section on the ovarian steroidogenesis. *Neuroimmunomodulation* 1999; 6: 293-9.
21. Bussmann LE, Deis RP. Studies concerning the hormonal induction of lactogenesis by prostalglandin F2a in pregnant rat. *J Steroid Biochem* 1979; 11: 1485-9.
22. Felten DL, Ackerman KD, Wiegand SJ, Felten SY. Noradrenergic sympathetic innervation of the spleen: I. Nerve fibers associate with lymphocytes and macrophages in specific compartments of the splenic white pulp. *J Neurosci Res* 1987; 18: 28-36.
23. Ackerman KD, Felten SY, Dijkstra CD, Livnat S, Felten DL. Parallel development of noradrenergic innervation and cellular compartmentation in the rat spleen. *Exp Neurol* 1989; 103: 239-55.
24. Sosa ZY, Casais M, Rastrilla AM, Aguado L. Adrenergic influences on coeliac ganglion affect the release of progesterone from cycling ovaries. Characterization of an in vitro system. *J Endocrinol* 2000; 166: 307-18.
25. Cannon JG, Dinarello CA. Increased plasma interleukin-1 activity in women after ovulation. *Science* 1985; 227: 1247-54.
26. Polan ML, Carding S, Loukides J. Progesterone modulates interleukine-1b (IL-1b) mRNA production by human pelvic macrophages. *Fertil Steril* 1988; 50: 54-67.
27. Ness JM, Kasson BG. Stimulation of rat granulosa cell progesterone production but not other differentiated functions by a splenocyte-derived factor. *J Reprod Fert* 1995; 105:125-33.
28. Kawamura N, Tamura H, Obana S, Wenner M, Ishikawa T, Nakata A, Yamamoto H. Differential effects of neuropeptides on cytokine production by mouse helper T cell subsets. *Neuroimmunomodulation* 1998; 5: 9-15.
29. Bracci Laudiero L, Aloe L, Stenfors C, Tirassa P, Theodorsson E, Lundberg T. Nerve growth factor stimulates production of neuropeptide Y in human lymphocytes. *Neuroreport* 1996; 7: 484-8.
30. Gorospe WC, Hughes FM, Spangelo BL. Interleukin-6: effects on and production by rat granulosa cell in vitro. *Endocrinology* 1992; 130: 1750-2.
31. Gorospe WC, Kasson BG. Lymphokines from concanavalin A-stimulated lymphocytes regulate rat granulosa cell steroidogenesis in vitro. *Endocrinology* 1988; 123: 2462-71.
32. Rivier C. Influence of immune signals on the hypothalamic-pituitary axis of the rodent. *Front Neuroendocrinol* 1995; 16: 151-82.