Chapter 4

Influence of Neuropeptide – Glutamic Acid-Isoleucine (NEI) on LH Regulation

María Ester Celis

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/48672

1. Introduction

Neuropeptide- glutamic acid isoleucine (NEI) is a peptide related to reproduction. Although it also has an important function on behavior [2-6], we will focus here on the relationship between NEI and LH.

NEI is derived from the precursor pre- prohormone named pp melanin-concentrating hormone (pp-MCH). This precursor also gives rise to melanin concentrating hormone (MCH) and to neuropeptide-glycine-glutamic acid (NGE) [7-9].

Some studies have suggested a role of NGE at the level of the hypothalamus. For instance, NGE increases the number of neurofilaments and the production of synaptophysin in rat neurons within 18 days of development [10-12].

Immunoreactivity and mRNA expression of both MCH and NEI have been observed in certain regions of the central nervous system. The first region is the diencephalon including the rostralmedial part of the zona incerta, later refer to as the incerto-hypothalamic area (Ihy) by Sita et al. [13]; the three subdivisions (anterior, tuberal and posterior) of the lateral hypothalamus (LHA); the area between the dorsomedial and ventromedial nuclei of the hypothalamus, which Swanson [14] designated as the internuclear area, the anterior periventricular nucleus; and the dorso medial aspects of the tuberomammillary complex. The second region includes the olfatory tubercle, located in the basal forebrain. The third region includes the paramedian pontine reticular formation in the pons [7]. It is important to note that the highest concentration of MCH/NEI ir cells is found in the Ihy and the LHA. We found nearly all the cells in these two regions were immureactive for both MCH and NEI (mean +-S.E.M, 96 +-3%) (Fig.1 and 2)

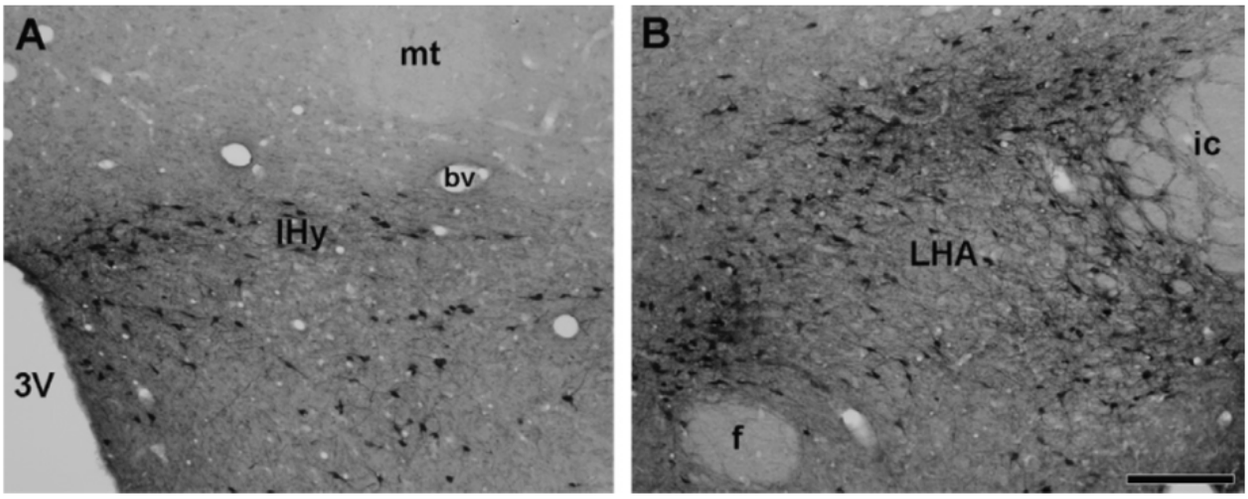

Figure 1. Diencephalic distribution of NEI-immunoreactivity-containing cells. Brightfield photomicrographs of immunoperoxidase material stained for NEI-ir. (A) NEI-ir-containing cells in the incerto-hypothalamic area. (B) NEI-ircontaining cells in the lateral hypothalamic area. Abbreviations: mt, mammilothalamic tract; bv, blood vessels; IHy, incerto hypothalamic area; 3v, third ventricle; ic, internal capsule; LHA, lateral hypothalamic area; f, fornix. Bar = 200 mm. - Reproduced from Bittencourt and Celis, 2008, with permission from Peptides (29:1441-50).

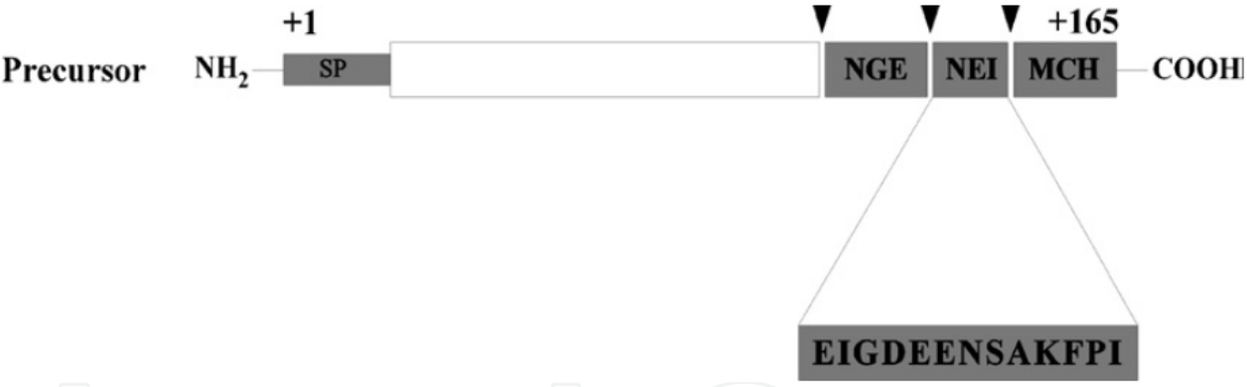

Figure 2. Deduced structure of rat prepro-MCH. The relative positions of the amino (NH2) terminal signal peptide (SP), and the putative MCH, NEI and NGE sequences at the carboxy (COOH) terminus are indicated. The amino acids sequence of NEI is expanded below it. The putative proteolitic processing sites are marked with arrowheads. - Reproduced from Bittencourt and Celis, 2008, with permission from Peptides (29:1441-50).

Peptides such as NGE, NEI and MCH are highly conserved among vertebrates, being abundant and widely distributed in the brain, suggesting that they could be performing important physiological functions. NEI is a 13 aminoacid peptide and has an extensive distribution in the central nervous system (CNS), acting as a neurotransmitter or neuromodulator [15-17]. Neurotranmitter NEI induces grooming behavior, locomotor activity, and stimulates sexual receptivity in female rats [1-6]. NEI injections can modify the levels of noradrenaline and dopamine in specific areas of the brain [4], and earlier studies demonstrated that MCH injection into the preoptic area or median eminence induces

luteinizing hormone (LH) secretion. Subsequent studies have shown that modified circulating hormonal levels might modulate ppMCH neurons [18] It has been reported that treatment with 17β-estradiol increases MCH and NEI immunoreactivity throughout the entire diencephalon of ovariectomized cynomologous monkeys [18]. Similar effects were observed in ovariectomized rats receiving no gonadal estradiol treatment [19]. Levels of ppMCH mRNA are only increased in the medial zone incerta (ZI) [15], which houses the A13 group, a collection of dopaminergic neurons that have been previously demonstrated to play a stimulating role in gonadotropin release [20].

More recently, various studies have explored the potential mechanisms by which MCH induces LH secretion [21-27]. Surprisingly, few have examined the role played by NEI in this process.

2. Ovarian steroids effects

Viale et al (1999) proposed a central role of the MCH/NEI neuronal system in the regulation of reproductive functions in rats [18]. In fact, the MCH/NEI system of immunoreactive fibers and terminals that is encountered in hypothalamic areas such as the medial preoptic area (MPOA), is well known to be involved in the control of pre-ovulatory LH surge. These authors studied the effects of the treatment with estrogen on the immunoreactivity of NEI and MCH in OVX animals, using the non-human primate (*M. Fasscicularis*). A slight increase of MCH-IR after 30 days post treatment with estrogen, along with a concurrent significant rise in NEI-IR was observed. A three-fold increase in MCH and NEI –Ir was seen at 72 h post estrogen treatment, compared with the amount of both peptides-ir at 48h post treatment. These facts suggest the possible involvement of these peptides in the regulation of the pre-ovulatory mid- cycle LH surge in primates [19].

The MCH receptors are classified into five subtypes: MC1-R to MC5-R [28]. MCH stimulates gonadotropin-releasing hormone (GnRH) release from hypothalamic explants, and it is interesting to note that MCH affects the release of LH [23] in the female rat. When MCH was injected bilaterally into the rostral preoptic area (rPOA) or medial preoptic area (mPOA) of estrogen-primed ovariectomized rats, LH release was stimulated. Two MCH receptors are involved in the MCH effect. The stimulatory action of MCH in the rPOA was inhibited by administration of antagonists for either MC-1 R or MC-5R, indicating that both ones, MC-1R and MC-5 are involved in the central control of GnRH release by MCH [19-27].

3. The effect of neuropeptide EI on LH regulation

For this study we used male and female rats, aged 10-14 weeks which were bred in our laboratory and maintained with food and water ad libitum, with a cycle of 14h/ light/10h dark and a temperature controlled environment (22±2ºC). The animal procedures were consistent with the standards established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996) and the AmericanVeterinarian Guidelines of Eutanasia.

The first evidence, on the effects of NEI on LH regulation was provided by Attademo et al [1], using male and ovariectomized rats treated with estrogen benzoate (10 ug) and low doses of progesterone (40ug). These animals revealed the following: when male rats were treated with intraventricular injections of NEI (1ug/1ul), the peptide induced an increase of serum LH concentration throughout the entire period studied (10-90 min). At 90 min the serum LH slightly decreased, possibly signaling initiation of the recovery of normal LH serum levels. Control rats injected with artificial cerebrospinal fluid showed practically no changes on serum LH concentrations (Fig. 3). It was also possible to see the NEI effect on ovariectomized female rats treated with estrogen plus progesterone, by using a low dose of progesterone to permit the visualization of modifications in the LH surge. Again, the neuropeptide increased LH release compared with control animals (Fig.4)

The fact that the effect of NEI may be mediated by the noradrenergic system must be taken into consideration. The peptide is known to modify DA and NA in the nucleus accumbens and caudate putamen during grooming behavior and locomotion activity [4]. As NEI behaves similarly to α-MSH, it is important to note that there is some relation between MCH-NEI and α-MSH, indicating that all three peptides are associated in a complex inter-relationship [1].

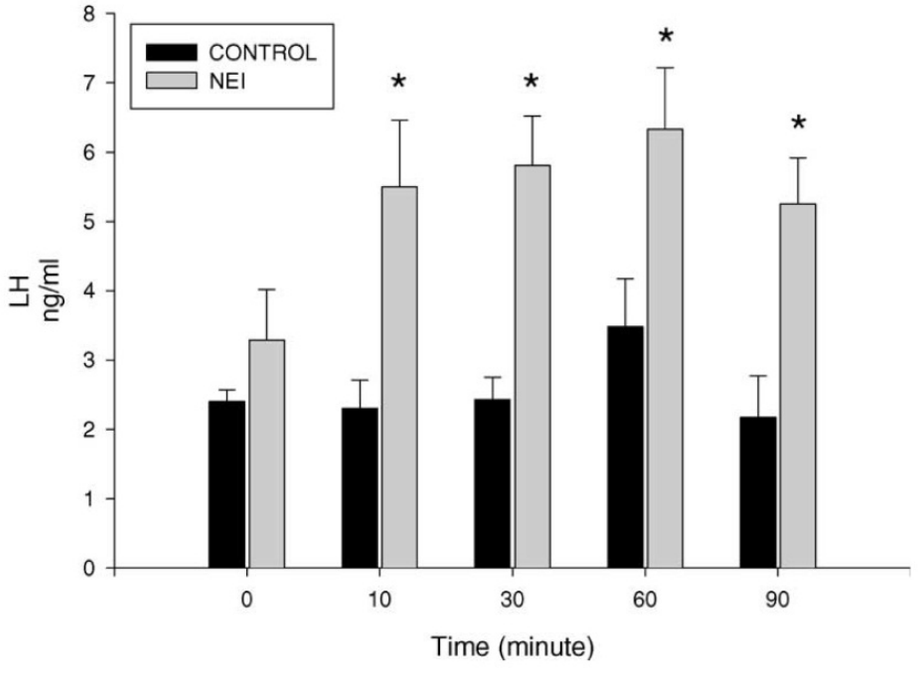

Figure 3. Effect of icv administration of NEI or ACSF (controls) on plasma LH concentration in male rats. Blood samples were collected at 0 (before NEI or ACSF injection), 10, 30, 60 and 90 min post-injection. Bars represent the mean ± S.E.M. $*P < 0.05$ compared to controls. - Reproduced from Attademo et al, 2004, with permission from Peptides.

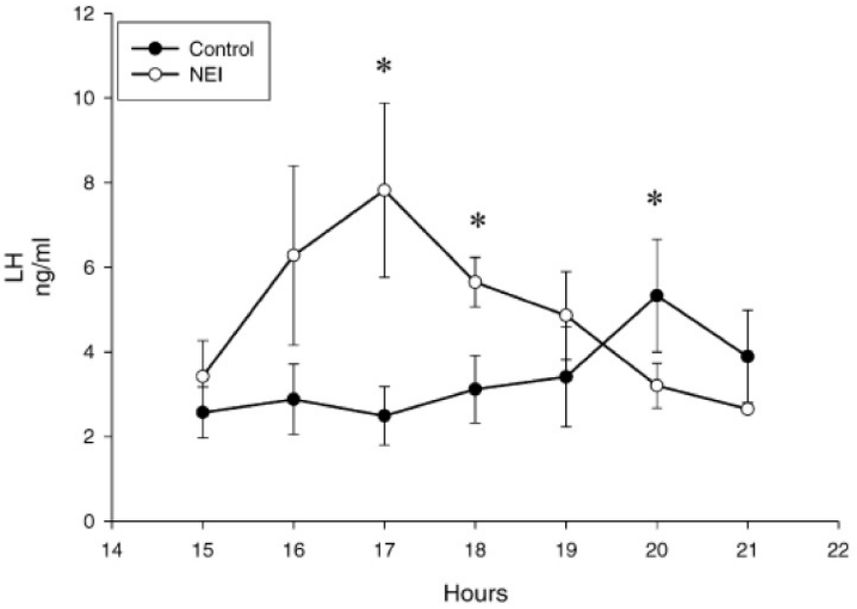

Figure 4. Time course of LH release in CHR-OVX-EB-progesterone treated female rats in the presence (◦) or absence (•) of NEI. CHR-OVX rats were subcutaneously injected with 10 ug EB and 3 days later with 40 ug progesterone at 13:00 h. On the same day of the progesterone injection, the animals were injected icv 1 ug/ul of NEI or ASCF (controls) at 12:00 and 14:00 h. Blood samples were obtained between 15:00 and 21:00 h via the jugular vein, and the plasma levels of LH were measured. The plotted values represent the mean ± S.E.M. ($n = 6$). $*P < 0.05$ compared to controls. - Reproduced from Attademo et al, 2004, with permission from Peptides.

4. Distribution of NEI immunoreactivity

In this study, we described the anatomical substrate underlying the NEI effect of inducing LH secretion, using techniques of double and triple label immunohitochemistry, as well as dual label immunofluorescence. A group of female rats were perfused on day 15 postovariectomy; a second group received10 μg of estradiol benzoate and were perfused two days later and a third group received 10 μg of estradiol benzoate and two days later 40 μg of progesterone and were perfused 5 h after treatment. To mimic the manipulation of the animals, we used a fourth group of ovariectomized rats treated with sesame oil, and also used female intact rats at proestrus and diestrus [28].

Using these techniques, we were able to obtain the following results:

NEI-ir neurons were observed in the medial ZI, in the perifornix at the tuberal hypothalamic level and in the lateral hypothalamus. Fibers were distributed throughout the forebrain, including areas related to reproductive control and LH secretion. We observed a dense number of NEI-ir fibers in the medial septal nucleus, the diagonal band of Broca, the environs of OVLT, the preoptic area and in the internal layer of the median eminence (Fig. 5).

All fibers seen in these areas show varicosities and terminal-like structures. NEI and terminal-like structures were in close apposition with portal blood vessels and GnRH neurons expressing Fos (Fig. 6 A and B)

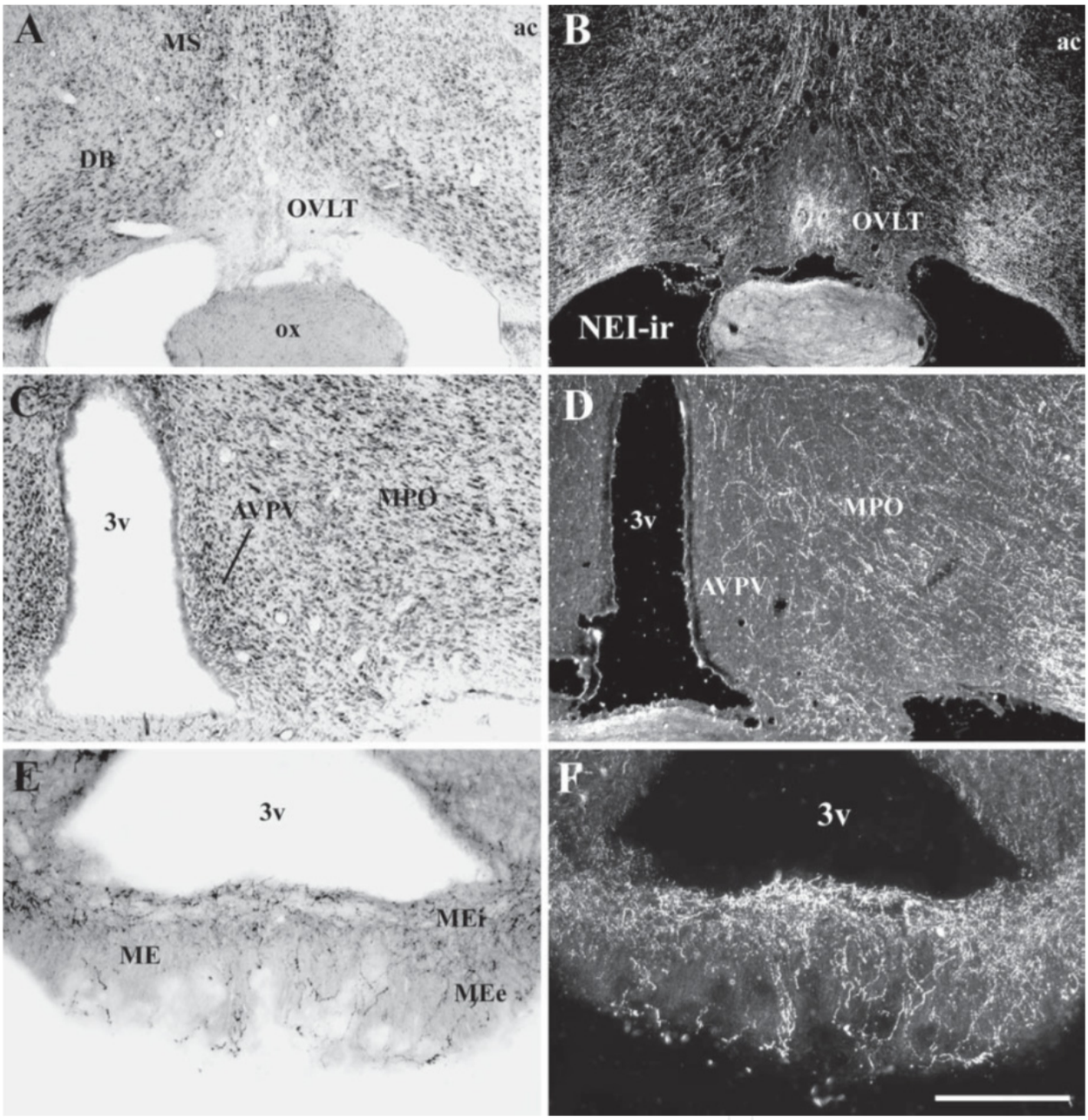

Figure 5. Distribution of NEI-ir fibers in forebrain areas related to reproduction. Bright-field photomicrographs of reference sections with thionine staining showing the OVLT (**A**) and AVPV (**C**). Dark-field photomicrographs showing the distribution of NEI-ir fibers in the environs of the OVLT (**B**) and in the AVPV (**D**). **E** Bright-field photomicrograph showing the distribution of NEI-ir fibers in the median eminence (ME). **F** Dark-field photomicrograph of the same section showing the distribution of NEIir fibers in the ME. MEi = Internal layer of the median eminence; MEe = external layer of the median eminence; ox = optic chiasm; 3v = third ventricle; ac = anterior commissure; DB = nucleus of the diagonal band; MPO = medial preoptic nucleus; MS = medial septal nucleus. Scale bar: 400 μm (**A–D**), 200 μm (**E** , **F**). - Reproduced from Attademo et al, 2006, with permission from Neuroendocrinology.

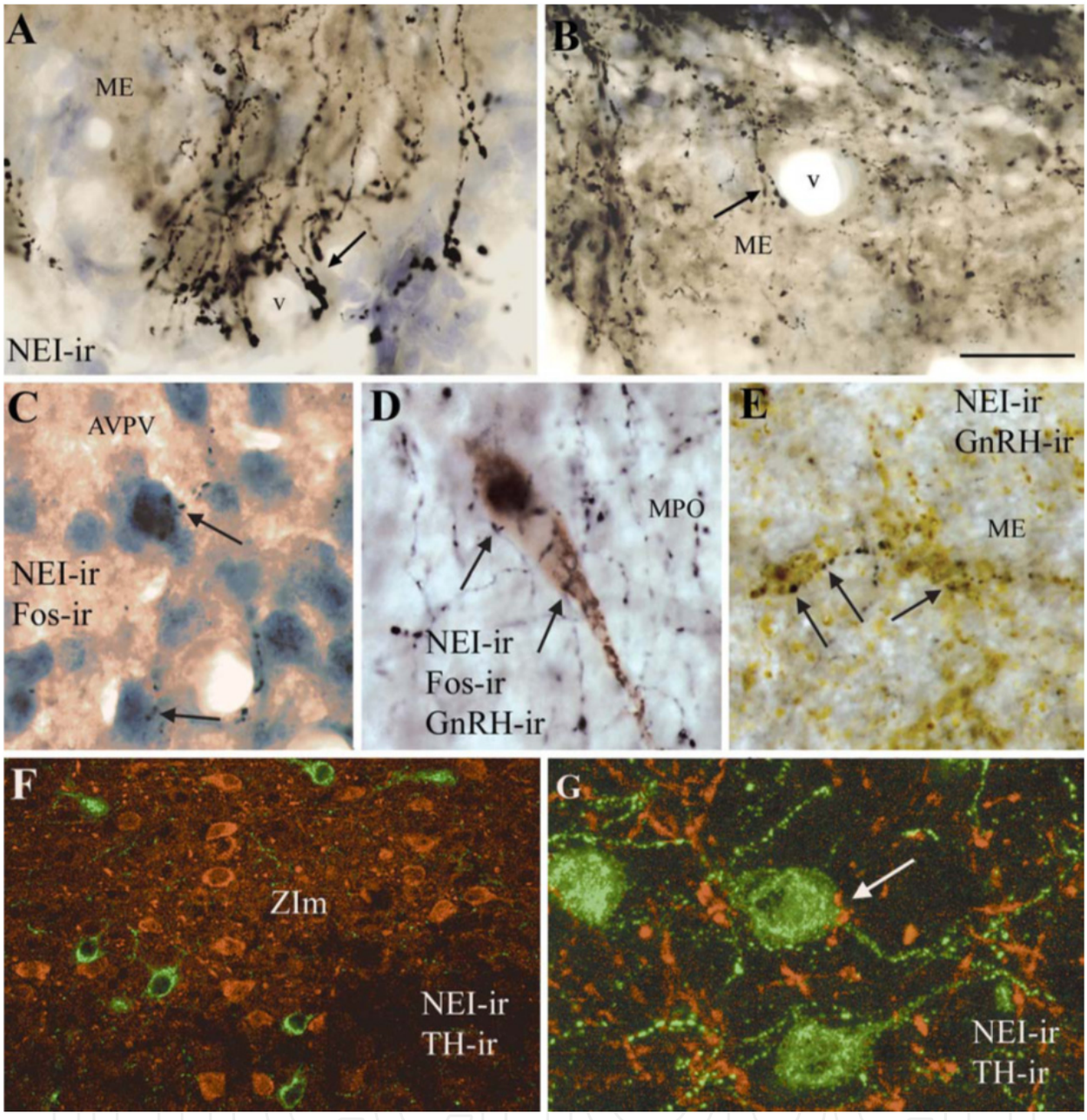

Figure 6. Alternative pathways for NEI induction of LH secretion. **A** , **B** Bright-field photomicrographs showing NEI-ir varicosities and terminal-like structures in the median eminence (ME). Note the close proximity to blood vessels (arrows). **C** Bright-field photomicrograph showing NEI-ir varicosities in close apposition with AVPV neurons expressing Fos (Fos-ir) in the afternoon of the proestrus day (arrows). **D** Bright-field photomicrograph showing NEI-ir fibers in close apposition with GnRH-ir neurons expressing Fos in the afternoon of the proestrus day (arrows). **E** Bright-field photomicrograph showing NEI-ir varicosities (in black) in close apposition with GnRH-ir fibers (in light brown) in the ME. **F** Fluorescence photomicrograph showing the close association between NEI-ir (in green, AlexaFluor 488) and TH-immunoreactive (TH-ir, in red, AlexaFluor 594) neurons in the medial zona incerta (ZIm). **G** Fluorescence photomicrograph showing TH-ir fibers in close apposition with NEI-ir neurons in the ZIm (arrow). v = Blood vessel; MPO = medial preoptic nucleus. Scale bar: 50 μm (**A–E** , **G**); 100 μm (**F**). - Reproduced from Attademo et al, 2006, with permission from Neuroendocrinology.

5. NEI-ir fibers innervate GnRH and AVPV neurons expresing fos

We observed an increased expression of Fos immunoreactivity in the anteroventral periventricular nucleus (AVPV) neurons of rats perfused during the afternoon of the day of proestrus, as well as in ovariectomized rats treated with estradiol benzoate plus progesterone, as described by others [30, 31]. Close to a 10% of the AVPV neurons expressing Fos receive NEI fibers in close apposition. Very little Fos immunoreactivity was observed in rats perfused in the afternoon of the diestrus day, for either of the following conditions: ovariectomized treated with estradiol benzoate or ovariectomized treated with sesame oil. In addition, all groups, showed a sparse distribution of Fos immunoreactivity in the medial zone incerta and in the LHA, whereas co-localization of NEI fibers with any of these cells was not observed (Fig. 6 D).

It has been reported that Fos protein is expressed in a portion of GnRH neurons that are active in the afternoon of the proestrus day, as well as in the GnRH neurons of ovariectomized rats treated with estradiol benzoate plus progesterone [31-33]. Based on these results, we investigated the pattern of NEI innervations in areas that expressed GnRH neurons in these animals. Most of the neurons expressing Fos were found to be in the vicinity of the organum vasculosum of the lamina terminalis (OVLT) and of the preoptic area. NEI fibers were also found in the median eminence, and NEI and GnRH varicosities presented similar distribution, thus revealing close apposition between them. Immediately before and during LH surge (on the afternoon of the proestrus day or following treatment with estrogen plus progesterone), only a portion of the GnRH neurons expressed Fos [30,31,33,34-36].

AVPV, is a nucleus that also expresses Fos in the afternoon of the proestrus day and has been widely implicated in the control of reproduction [32], with Fos expression indicating neuronal response [36]. However, in rats perfused on the diestrus day or in ovariectomized rats receiving estradiol benzoate or sesame oil, we did not find Fos expression in GnRH neurons. The pattern of distribution of NEI-ir and GnRH ir in the median eminence, showed NEI fibers to be denser in the internal layer than in the external one. Nevertheless, in the external layer, NEI and GnRH varicosities presented similar distributions, revealing an apparent close apposition between them. In the present study, we labeled distinctive cell compartments by using dual and triple-label immunohistochemistry which revealed NEI-ir fibers to be innervating the Fos-positive neurons in the AVPV, as well as the GnRH neurons positive for Fos immunoreactivity. In these experiments we labeled distinct cell compartments (cytoplasm, nuclei and terminals) using various antisera, all raised in rabbit. In the control tests, the second or third antisera were omitted, and no reaction was evident. This indicates that the observed labeling of cell bodies, fibers or both was not the result of cross-reactivity of the secondary antibody. In addition, evaluation of the data under light microscopy at a high magnification revealed only a suggestion of synaptic contact (Fig 6 A-B).

6. NEI innervation of GnRH neurons

Our results indicated that NEI fibers were in close apposition with GnRH neurons expressing Fos in the afternoon of the proestrus day. Since Fos protein expression in GnRH

neurons increases in parallel with rises in the plasma LH levels [27, 31, 32], it can be assumed that these neurons project to the median eminence and induce LH secretion during proestrus. Therefore, we can suggest that NEI (through projections to a subset of GnRH neurons) modulated GnRH activity and, consequently, LH surges.

The role that NEI plays in GnRH secretion has not been investigated. However, NEI varicosities in some parts of the median eminence display a pattern of distribution similar to that of GnRH, revealing a possible effect on the modulation of GnRH secretion directly at the terminals. This may represent one of the mechanisms by which intracerebroventricular administration of NEI causes an increase in LH secretion (Fig. 6 E)

Experiments using in vitro preparations or intracerebroventricular injections have shown that various neurotransmitters can regulate gonadotropin release [38-40]. One such neurotransmitters is the cocaine- and amphetamine- regulated transcript (CART) peptide, which has been shown to increase the GnRH pulse amplitude in cycling female rats and to decrease GnRH pulse intervals in prepubertal rats [41-43]. These effects can be achieved through direct CART innervation of the GnRH neurons [39, 40]. Interestingly, in the medial ZI and lateral hypothalamus, MCH/NEI neurons coexpress CART [44, 46]. In the present study, we did not explore the origins of NEI innervation of GnRH neurons or areas related to reproductive behavior. However, it is intriguing that the number of NEI-ir neurons in ovariectomized rats was increased only in the medial ZI, a brain region that projects to the AVPV and GnRH- containing areas [47,48], as well as to the circumventricular organs, probably including the median eminence [44, 45]. This result is in agreement with those of other studies in which ppMCH mRNA expression was found to be greater in the medial ZI of untreated ovariectomized rats than in that of ovariectomized rats primed with estradiol benzoate or estradiol benzoate plus progesterone.

7. In vitro studies

Taken into account the above results, we decided to investigate whether NEI could act directly at the pituitary level by modulating hormone secretion. With this purpose the effects of NEI were studied in pituitary cell cultures from female rats on the release of several pituitary hormones (GH, LH and prolactin). Furthermore, the ability of NEI to activate pituitary cells was evaluated by electron microscopy and immunocitochemistry [49], and finally, the ability of NEI to potentiate GnRH-induced LH release was tested. The study of the effects of physiological stimuli involved in the regulation of pituitary hormone secretion was facilitated by the availability of a system consisting of a suspension of single dispersed pituitary cells, in which the cell functions were essentially the same as in situ [50]. For this study we used female rats in order to obtain a primary culture. The results were as follows:

NEI induced a fast release of LH in the culture cell media. In addition, there were differences in the LH levels obtained for the various time periods of hormonal stimuli assayed, which were closely associated with the doses applied. The lowest dose of NEI (100x 10^{-8}M) induced a significant increase of LH secretion after 2h of stimulus, achieving maximum response after 4h of NEI treatment. At this time, the LH levels almost reached a

five-fold increase over controls and then maintained these values (Fig.7). In spite of NEI being effective in stimulating LH secretion, none of the assayed doses were capable of significantly promoting FSH secretion from gonadotrophs. In addition, no significant increases were observed on prolactin or GH secretion, at doses ranging from $100x10^{-8}$ to $400x10^{-8}$ M NEI in primary cell cultures, thus confirming the specificity of NEI stimuli on LH secretion (Table 1). To determine whether or not NEI was able to synergize with GnRH in stimulating LH release, pituitary cells were simultaneously incubated with GnRH (0.1 or $1x10^{-9}$M) and different concentrations of NEI (1, 10 or $100x10^{-8}$M) for 3 h and then the media was collected and tested for LH by RIA (Fig. 8). Although NEI at the dose of $10x10^{-8}$M had no effect on LH secretion, GnRH $1x10^{-9}$M plus NEI $10x10^{-8}$M induced a slight but significant increase in LH concentrations (16%; $p< 0.01$). A combined treatment with the highest doses of both NEI and GnRH significantly stimulated the secretory response, which was more effective than that observed with the same dose of GnRH alone [49] (Fig.8).

When pituitary cells from female rats were cultured, different types of secretory cells were observed (Fig. 9). These were identifiable by their ultra structural characteristics, essentially by the profile of the secretory granules, which constituted a distinctive feature. The most frequent populations observed were lactotroph and somatotroph cells, which were in close contact with gonadotrph cells. In the control group, the lactotroph and somatotroph cells have numerous polymorphic and round mature secretory granules respectively. These also had high electron densities and were stored in the cytoplasm. The gonadotroph were

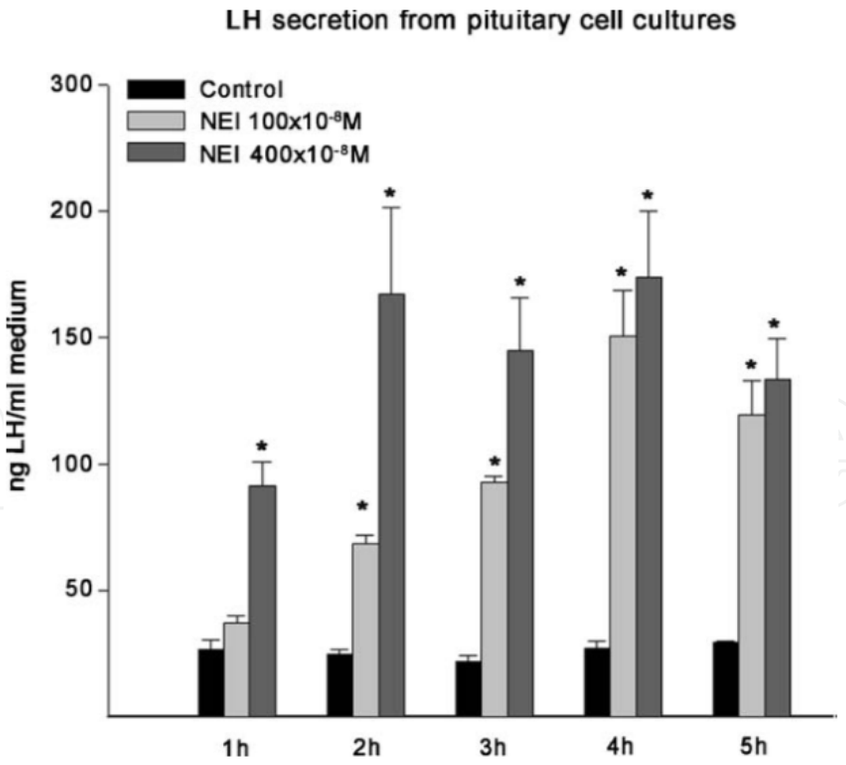

Figure 7. Time-course study of the effects of NEI on LH secretion in the culture media. The cell cultures were treated with NEI 100 or 400 x 10^{-8} M for 1–5 h, in serum free conditions. The data are represented as mean ± S.E.M. of three independent experiments. Data were evaluated by the ANOVA–Fisher test; *$p < 0.01$ vs Control group. – Reproduced from De Paul et al, 2009, with permission from Peptides.

	Time exposition	Control Mean ± S.E.M.	NEI 100 × 10^{-8} M Mean ± S.E.M.	NEI 400 × 10^{-8} M
FSH secretion	1 h	32.41 ± 4.93	38.16 ± 1.05	38.1 ± 3.30
	2 h	32.24 ± 3.22	38.46 ± 1.70	31.92 ± 5.59
	3 h	28.83 ± 3.15	36.23 ± 1.82	24.65 ± 1.35
	4 h	25.80 ± 2.22	29.53 ± 2.09	19.88 ± 1.36
	5 h	28.30 ± 3.18	28.91 ± 3.15	22.35 ± 1.30
PRL secretion	1 h	839.42 ± 47.96	998.65 ± 42.09	926.64 ± 96.40
	2 h	1079.81 ± 56.38	1204.50 ± 93.25	951.60 ± 69.38
	3 h	1182.30 ± 92.82	1338.38 ± 141.05	936.41 ± 27.40
	4 h	1567.68 ± 95.27	1703.32 ± 26.56	1373.41 ± 157.41
	5 h	1537.53 ± 66.10	1736.56 ± 129.94	1308.8 ± 56.86
GH secretion	1 h	395.20 ± 56.05	309.13 ± 36.45	502.79 ± 26.09
	2 h	485.51 ± 49.54	426.75 ± 42.88	533.82 ± 36.48
	3 h	522.03 ± 70.54	585.64 ± 37.47	508.72 ± 49.67
	4 h	740.07 ± 96.60	729.82 ± 76.28	618.08 ± 71.91
	5 h	730.90 ± 38.26	897.58 ± 72.80	676.75 ± 66.53

Table 1. Time-course study of the effects of NEI on FSH, PRL and GH secretion accumulated in the culture media (ng/ml of culture medium). Pituitary cells were treated with NEI 100 or 400×10^{-8}M for 1–5 h. The data are shown as the mean ±S.E.M. of three independent experiments and were evaluated by the ANOVA–Fisher test.

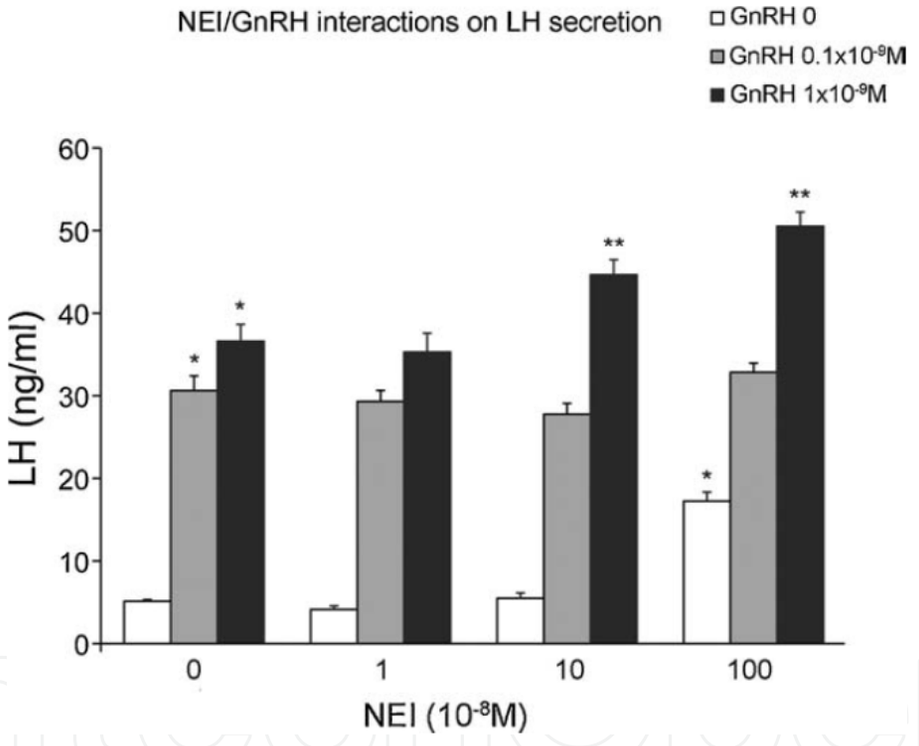

Figure 8. NEI and GnRH combined treatments on LH secretion. The presence of 10 and 100 x 10^{-8} M NEI in the culture media for 3 h promoted a significant increase in LH release stimulated by GnRH 1 x10^{-9} M. Data are shown as the mean ± S.E.M. of three independent experiments. ANOVA–Fisher test; *p < 0.01 vs control group; **p < 0.01 vs GnRH 1 x 10^{-9} M. – Reproduced from De Paul et al, 2009, with permission from Peptides.

characterized by a conspicuous accumulation of round secretory granules of two different sizes and by electron densities in the cytoplasm. The most abundant granules were about 150nm in diameter and filled with homogenous material, whereas the others less frequent but larger in size (about 400nm) (Fig. 10 A and B).Stimulation with NEI (100 x 10^{-8} and 400 x 10^{-8}) for 2 and 4 hour, promoted several subtle structural changes, particularly in the

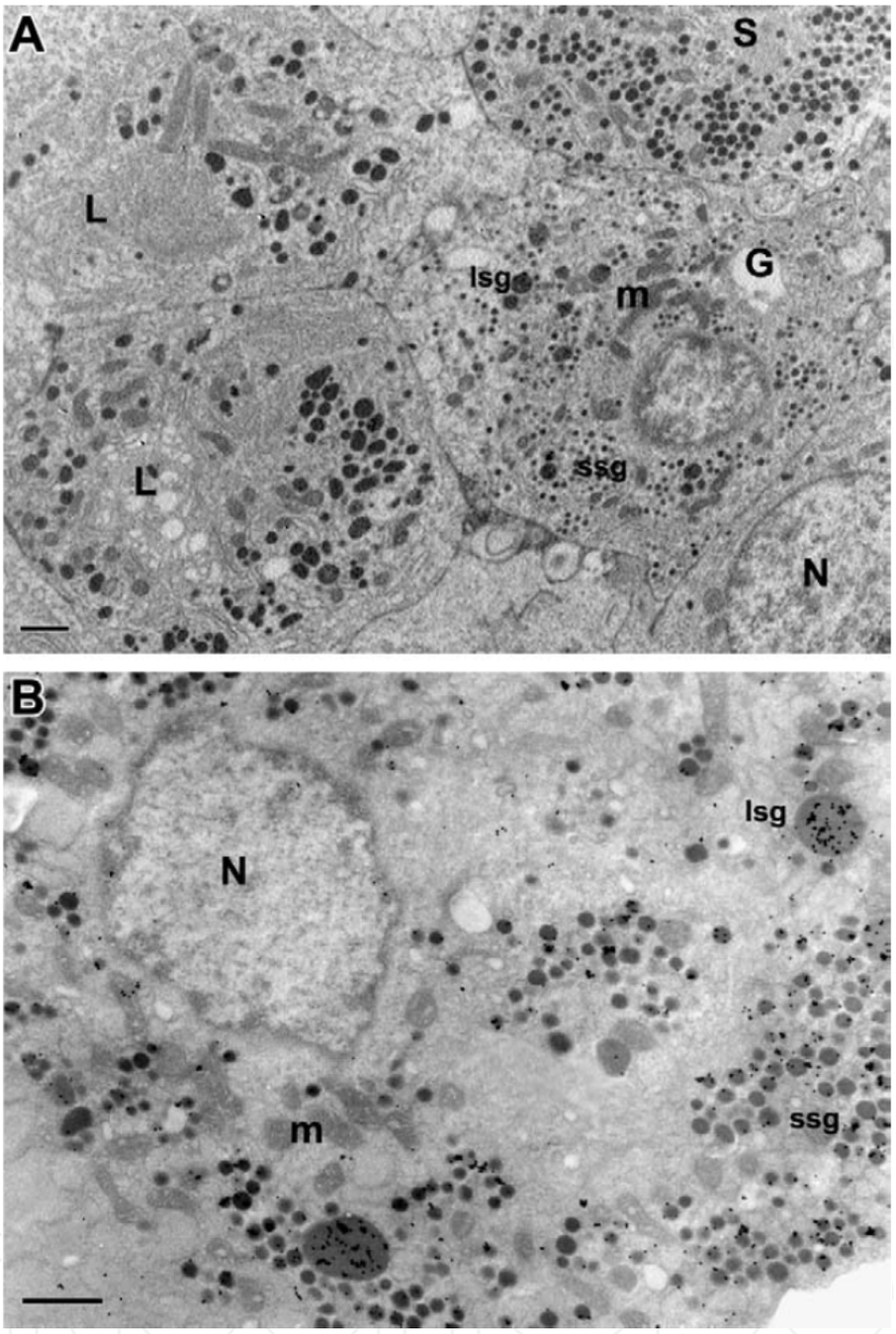

Figure 9. (A) Electron micrograph of a pituitary cell culture from control rats illustrating different pituitary cell populations. Two lactotroph cells (L) exhibit an accumulation of large and polymorphic mature secretory granules (about 500– 900 nm in diameter) in the cytoplasm. The somatotroph cell (S) can be easily recognized by the mature, round GH secretory granules ranging from 200 to 350 nm in diameter and scattered throughout the cytoplasm. In close contact with both secretory cell types, a gonadotroph cell (G) displays small (ssg) and large (lsg) round secretory granules (about 150 or 400 nm in diameter respectively, with different electron densities, homogeneously disseminated in the cytoplasm (m = mitochondria, N = nucleus). Bar: 1 μm. (B) Gonadotroph cell specifically immunostained for LH. The cytoplasm shows a noticeable accumulation of characteristic small (ssg) and large (lsg) round secretory granules (m = mitochondria, N = nucleus). Bar: 0.5 μm. – Reproduced from De Paul et al, 2009, with permission from Peptides.

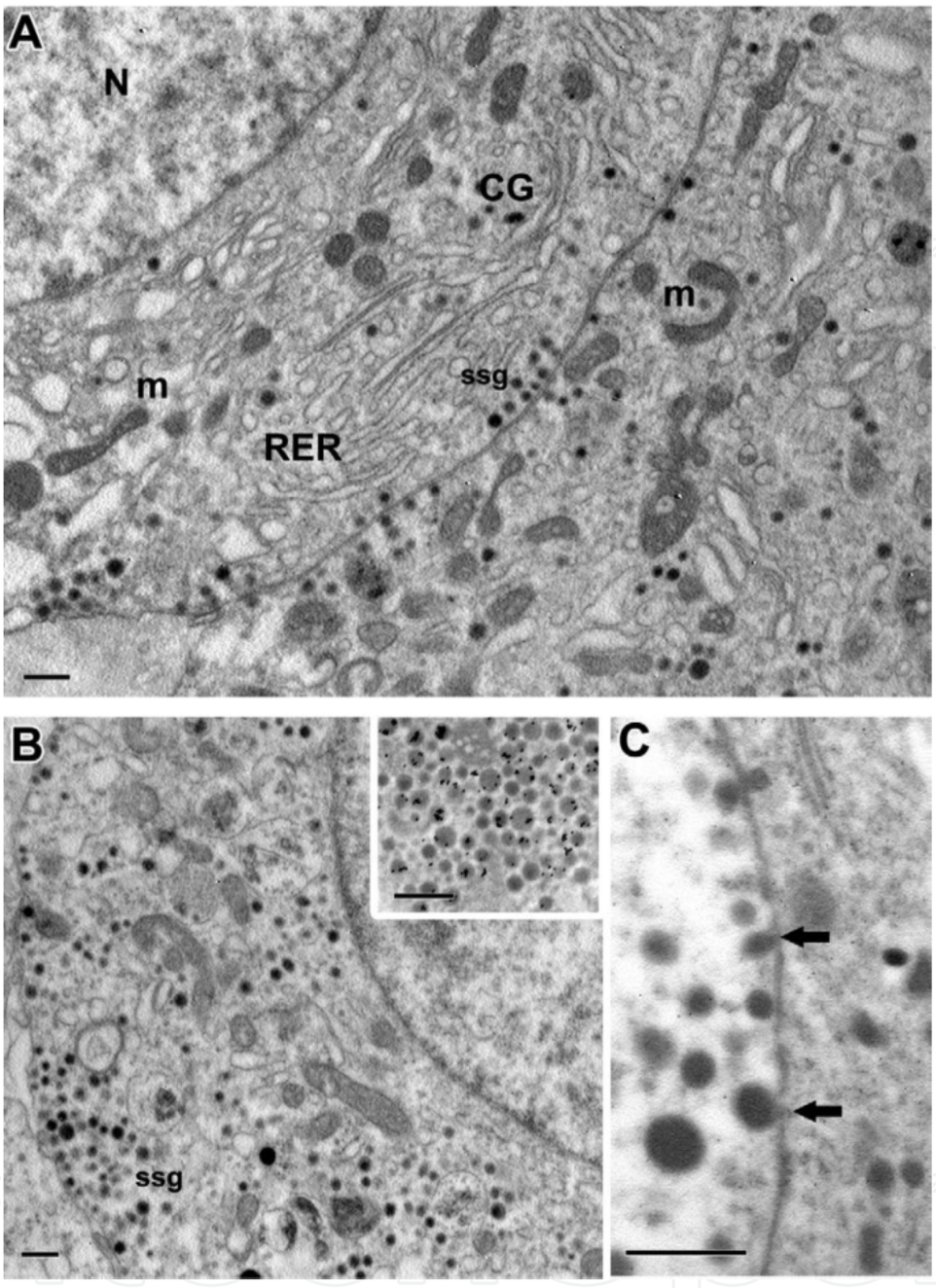

Figure 10. (A) Electron microscopy of two cultured gonadotroph cells treated with 400 x 10^{-8} M NEI for 4 h. The cytoplasm contains a remarkably well developed rough endoplasmic reticulum (RER) and Golgi complex (GC) and also scarce small secretory granules (ssg) that are mostly in contact with the plasma membrane. Bar: 0.5 μm. (B) Electron micrograph of a cultured gonadotroph cell (G) after exposition to 100 x 10^{-8} M NEI for 2 h which is shown exhibiting small secretory granules (ssg) mobilized toward the plasmalemma, where they will then be subsequently discharged by exocytosis. Bar: 0.5 mm. Inset: small round secretory granules from a gonadotroph cell specifically identified by immunocytochemistry for LH. Bar: 0.5 mm. (C) Detail of two adjacent gonadotroph cells after NEI treatment displaying evidence of secretory activity. The secretory granules are aligned alongside the cell membrane and are in the process of exocytosis (arrows). Bar: 0.5 μm. – Reproduced from De Paul et al, 2009, with permission from Peptides.

gonadotroph cell population. For this cell type, the most prominent changes consisted of a striking development of the rough endoplasmic reticulum (RER) and Golgi complex (Fig 10B) when compared to the control group. Many secretory granules were located to the cell membrane and presented images of exocytosis after NEI treatment (Fig. 10 C) Other pituitary cell populations, the lactotrophs, thyrotrophs and somatotrophs, did not exhibit any features indicating a significant activation of hormone release after NEI treatment.

The present results were the first demonstration of a specific and direct action of NEI in cultured pituitary cells without modifying other pituitary hormones. Moreover, the analysis of the electron microscope images taken 2 and 4h after NEI treatment was indicative of the stimulation of LH release occurring at these times.

From the present study, it is possible to conclude that NEI is effective when injected into the brain to release LH in male and female rats. The anatomical substrate underlying this effect was identified using combined methods of immunohistochemistry. A schematic representation of the proposed pathways by which NEI participates in LH secretion is depicted in Figure 11.

NEI is also capable of inducing a marked release of LH without modifying the other pituitary hormones in the pituitary cultured cells. There is an interaction between NEI and GnRH in vivo and in vitro.

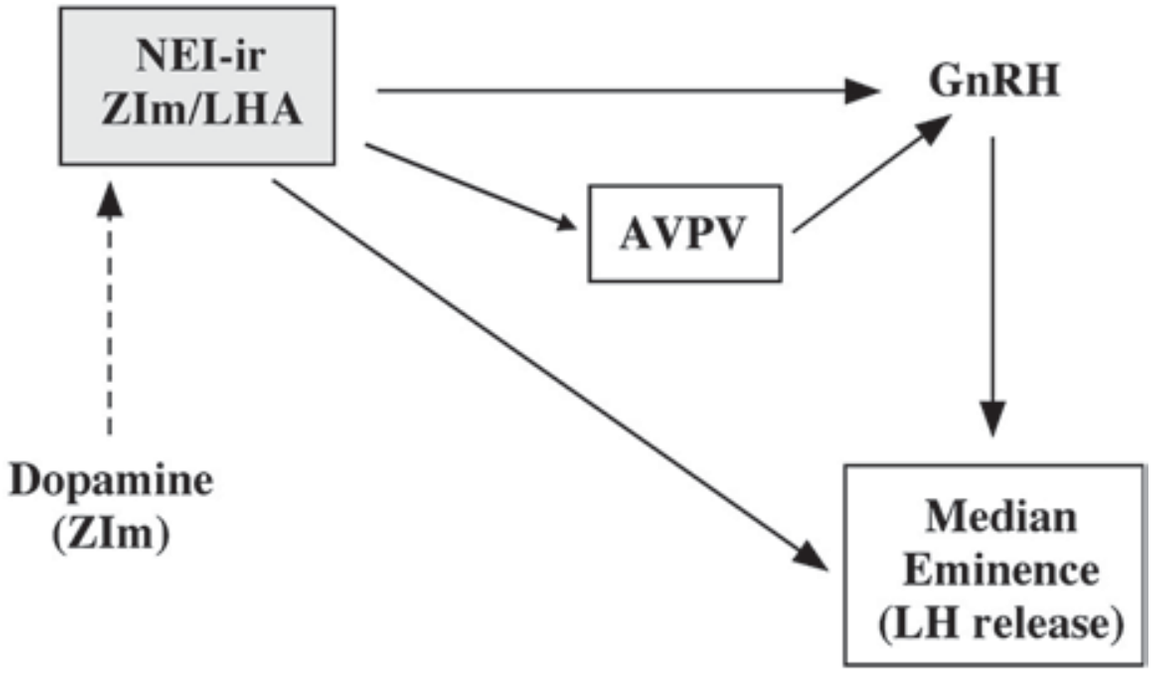

Figure 11. Schematic representation of the proposed pathways by which NEI participates in LH secretion. NEI-ir neurons in the medial zona incerta (ZIm) or LHA receive dopaminergic innervation from TH neurons located in the ZIm and project directly to the median eminence or to GnRH neurons in the preoptic area. In addition, NEI might modulate LH secretion by innervating the AVPV. – Reproduced from Attademo et al, 2006, with permission from Neuroendocrinology.

Author details

María Ester Celis
Laboratorio de Ciencias Fisiológicas, Cátedra de Bacteriología y Virología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Santa Rosa, Córdoba, Argentina

Acknowledgement

First of all I wish to thank to Dr. Paul Hobson for critical reading of this manuscript. I also wish to thank Dr. Jorge Paván, Miss Laura Alazraki and Miss Marina Juarez for editing the manuscript. Thanks for the grants from CONICET; SECyT and FONCyT. MEC is member of CONICET.

8. References

[1] Attademo AM, Sanchez-Borzone M, Lasaga M, Celis ME. Intracerebroventicular injection or neuropeptide El increases serum LH in male and female rats. Peptides 2004; 25:1995-9

[2] Berberian V, Sanchez MS, Celis ME. Participation of the cholinergic system in the exesesive grooming behavior induced by neuropeptide (N) glutamic acid (E) isoleucine (I) amide (NEI). Neurochem Res 2002; 27:1713-1717.

[3] Sanchez M, Baker BI, Celis M: Melanin-concentrating hormone (MCH) antagonizes the effects of alpha-MSH and neuropeptide E-I on grooming and locomotor activities in the rat. Peptides 1997; 18:393-396.

[4] Sanchez MS, Barontini M, Armando I, Celis ME: Correlation of increasing grooming and motor activity with alterations in nigrostriatal and mesolimbic catecholamines after alpha melanocortin and neuropeptide glutamine-isoleucine injection in the tegmental area. Cell Mol Neurobiol 2001; 21(5):523-33.

[5] Sánchez- Borzone M, Attademo AM, Baiardi G, Celis ME: Effect of β- adrenoceptors on the behavior induced by the neuropeptide glutamic acid isoleucine amide. Eur J Pharmacol 2007; 568:186-91.

[6] Gonzales MI, Baker BI, Hole DR, Wilson CA: Behavioral effects of neuropeptide E-I (NEI) in the female rat: Interactions with α- MSH, MCH and dopamine. Peptides 1998;19:1007-1016.

[7] Nahon JL, Presse F, Bittencourt JC, Sawchenko PE, Vale W. The rat melanin-concentrating hormone messenger ribonucleic acid encodes multiple putative neuropeptides coexpressed in the dorsolateral hypothalamus. Endocrinology 1989; 125:2056-65.

[8] Parkes DG, ValeW. Secretion of melanin-concentrating hormone and neorupeptide- EI from cultured rat hypothalamic cells. Endocrinology 1992;131:1826-31.

[9] Presse F, Nahon JL, Fischer WH, Vale W. Structure of the human melanin- concentrating hormone mRNA. Mol Endocrinol 1990;4:632-7.

[10] Nahon JL, Presse F, Bittencourt JC, Sawchenko PE, Vale W.The rat melanin-concentrating hormone messengerribonucleic acid encodes multiple putatives neuropeptides coexpressed in the dorsolateral hypothalamus. Endocrinology 1989;125:2056-65.

[11] ParkesDG.,Constracting actionsVale W. Secretion of melanin-concentrating hormone and neuropeptide-EI from cultured rat hypothamic cells. Endocrinology 1992; 131:1826-31.

[12] Parkes DG., Vale W Constrasting actions of melanin-concentrating hormone and neuropeptide –E-I on posterior pituitary function. Ann NY Acad Sci 1993;680:580-90.

[13] SitaLV, ElíasCF; bittencourt JC. Connectivity pattern suggests that incero-hypothalamic area belongs to the medial hypothalamic system. Neuroscience 2007;148(4):949-69.

[14] Swanson LW.Brainmaps: structure of the rat brain, 3rd edition, Academic Press; 2004

[15] Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon JL, Vale W, Sawchenko PE: The melanin-concentrating hormone system of the rat brain: an inmuno-and hybridization histochemical characterization. J Comp. Neurol 1992; 319:218-245.

[16] Presse F, Nahon J. Differential regulation of melanin- concentrating hormone gene expression in distinct hypothalamic areas under osmotic stimulation in rat. Neuroscience 1993,55:709-720.

[17] Bittencourt JC, Elias CF. Melanin- concentrating hormone and neuropeptide EI projections from the lateral hypothalamic area and zona incerta to the medial septal nucleus and spinal cord: a study using multiple neuronal traces. Brain Res 1998; 805(1-2):1-19.

[18] Viale A, Kerdelhue B, Nahon JL: 17beta-estradiol regulation of melanin-concentrating hormone and neuropeptide- E-I contents in cynomolgus monkeys: a preliminary study. Peptides 1999; 20:553-559.

[19] Murray JF, Baker BI, Levy A, Wilson CA: The influence of gonadal steroids on pre-promelanin-concentrating hormone m RNA in female rats. Neuroendocrinol 2000; 12:53-59.

[20] MacKenzie FJ, James MD, Wilson CA: Changes in dopamine activity in the zona incerta (ZI) over the rat oestrous cycle and the effect of lesion of the ZI on cyclicity: further evidence that the incerto- hypothalamic tract has a stimulatory role in the control of LH release. Brain Res 1988; 444:75-83.

[21] Murray JF, Adan RA, Walker R, Baker BI, Thody AJ, Nijenhuis WA, Yukitake J, Wilson CA: Melanin- concentrating hormone, melacortin receptors and regulation of luteinizing hormone release. J Neuroendocrinol 2000; 12:217-223.

[22] Murray JF, Hahn JD, Kennedy AR, Small CJ, Bloom SR, Haskell-Luevano C, Coen CW, Wilson CA: Evidence for stimulatory action of melanin-concentrating hormone on luteinising hormone release involving MCH1 and melanocortin-5 receptors. J Neuroendocrinol 2006; 18:157-167.

[23] Murray JF, Mercer JG, Adan RA, Datta JJ, Aldairy C, Moar KM, Baker BI, Stock MJ, Wilson CA: The effect of leptin on luteinizing hormone release is exerted in the zona incerta and mediated by melanin-concentrating hormone. J Neuroendocrinol 2000; 12:1133-1139.

[24] Chiocchio SR, Gallardo MG, Louzan P, Gutnisky V, Tramezzani JH: Melanin-Concentrating hormone stimulates the release of luteinizing hormone-releasing hormone and gonadotropins in the female rat acting at both median eminence and pituitary levels. Biol Reprod 2001; 64:1466-1472.

[25] Williamson- Hughes PS, Grove KL, Smith MS: Melanin concentrating hormone (MCH): a novel neutral pathway for regulation of GnRH neurons. Brain Res 2005; 1041:117-124.

[26] Gonzales MI, Baker BI, Wilson CA: Stimulatory effect of melanin-concentrating hormone on luteinising hormone release. Neuroendocrinology 1997; 66:254-262.

[27] Schlumberger SE, Talke- Messerer C, Zumsteg U, Eberle AN: Expression of receptors for melanin-concentrating hormone (MCH) in different tissues and cell lives. J Recept Signal Transd Res 2002; 22: 509-531.

[28] Attademo AM, Rondini TA, Rodrigues BC, Bittencourt JC, Celis ME, Elias CF. Neuropeptide glutamic acid-isoleucine may induce luteinizing hormone secretion via multiple pathways. Neuroendrocrinology 2006; 83:313-24.

[29] Hoffman GE, Lee WS, Attardi B, Yann V, Fitzsimmons MD. Luteinizing hormone releasing hormone neurons express c-fos antigen after steroid activation. Endocrinology 1990; 126:1736-1741.

[30] Hoffman GE, Smith MS, Verbalis JG: C-fos and related immediate early gene products as makers of activity in neuroendocrine systems. Front Neuroendocrinol 1993; 14:173-213.

[31] Le WW, Attardi B, Berghorn KA, Blaustein J, Hoffman GE: Progesterone blockade of a luteinizing hormone surge blocks luteinizing hormone-releasing hormone fos activation and activation of its preoptic area afferents. Brain Res 1997; 778:272-280.

[32] Le WW, Berghorn KA, Rassnick S, Hoffman GE: Periventricular preoptic area neurons coactived whit luteinizing hormone (LH) releasing hormone (LHRH) neurons at the time of the LH surge are LHRH afferents. Endocrinology 1999;140:510-519.

[33] Lee WS, Smith MS, Hoffman GE: Luteinizing hormone- releasing hormone neurons express fos protein during the proestrous surge of luteinizing hormone. Proc Natl Acad Sci USA 1990; 87:5163-5167.

[34] Wang HJ, Hoffman GE, Smith MS: Increased GnRH mRNA in the GnRH neurons expressing cFos during the proestrous LH surge. Endocrinology 1995; 136:3673-3676.

[35] Hoffman GE, Lyo D: Anatomical markers of activity in neuroendocrine systems: are we all "fos-ed out"? J. Neuroendocrinol 2002; 14:259-268.

[36] Bittencourt JC, Elias CF: Melanin- Concentrating hormone and neuropeptide EI projections from the lateral hypothalamic area and zona incerta to medial septal nucleus and spinal cord: a study using multiple neuronal tracers. Brain res 1998;805:1-9.

[37] Levine JE, Pau KY, Ramirez VD, Jackson GL: Simultaneous measurement of luteinizing hormone- releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. Endocrinology 1982; 111: 1449-1455.

[38] Negro-Vilar A, Ojeda SR, McCann SM: Catecholaminergic modulation of luteinizing hormone-releasing hormone release by median eminence terminals in vitro. Endrocrinology 1979; 104: 1749-1757.

[39] Rasmussen DD, Kennedy BP, Ziegler MG, Nett TM: Endogenous opioid inhibition and facilitation of gonadotropin-releasing hormone release from the medial eminence in vitro: potencial role of catecholamines. Endocrinology 1988; 123: 2916-2921.
[40] Bourguignon JP, Gerard A, Franchimont P: Direct activation of gonadotropin-releasing hormone secretion through different receptors to neuroexitatory amino acids. Neuroendocrinology 1989; 49: 402-408.
[41] Lebrethon MC, Vandermissen E, Gerard A, Parent AS, Junien JL, Bourguignon JP: In vitro stimulations of the prepubertal rat gonadotropin-releasing hormone pulse generartor by leptin and neuropeptide Y through distinct mechanism. Endocrinology 2000; 141: 1464-1469.
[42] Parent AS, Lebrethon MC, Gerard A, Vandermissen E, Bourguignon JP: Leptin effects on pulsatile gonadotropin releasing hormone secretion from the adult rat hypothalamus and interaction with cocaine and amphetamine regulated transcript peptide and neuropeptide Y. Regul Pept 2000; 92: 17-24.
[43] Lebrethon MC, Vandermissen E, Gerard A, Parent AS, Bourguignon JP: Cocaine and amphetamine-regulated-transcript peptide mediation of leptin stimulatory effect on the rat gonadotropin-releasing hormone pulse generator in vitro. J Neuroendocrinol 2000; 12: 383-385.
[44] Leslie RA, Sanders SJ, Anderson SI, Schuhler S, Horan TL, Ebling FJ: Appositions between cocaine and amphetamine-related transcript- and gonadotropin releasing hormone-immunoreactive neurons in the hypothalamus of the Siberian hamster. Neurosci Lett 2001; 314: 111-114.
[45] Rondini TA, Baddini SP, Sousa LF, Bittencourt JC, Elias CF: Hypothalamic cocaine and amphetamine-regulated transcript neurons project to areas expressing gonadotropin releasing hormone immunoreactivity and to the anteroventral periventricular nucleus in male and female rats. Neuroscience 2004; 125:735-748.
[46] Elias CF, Lee CE, Kelly JF, Ahima RS, Kuhar M, Saper CB, Elmquist JK: Characterization of CART neurons in the rat and human hypothalamus. J Comp Neurol 2001; 432:1-19.
[47] Broberger C: Hypothalamic cocaine- and amphetamine- regulated transcript (CART) neurons: Histochemical relationship to thyrotropin-releasing hormone, melanin-concentrating hormone, orexin/hypocretin and neuropeptide Y. Brain Res 1999;848:101-113.
[48] Hahn J, Coen C: Comparative study of the sources of neuronal projections to the site of gonadotropin- releasing hormone perikarya and to the anteroventral periventricular nucleus in female rats. J Comp Neurol 2006; 494: 190-214.
[49] De Paul AL, Attademo AM, Torres AI, Jahn GA, Celis ME. Neuropeptide glutamic-isoleucine (NEI) specifically stimulates the secretory activity of gonadotropes in primary cultures of female rat pituitary cells. Peptides 2009; 30: 2081-2087.
[50] De Paul Al, Pons P, Auki A, Torres A: Different behavior of lactotroph cell in response to angiotensin II and thyrotropin-releasing hormone. Cell Mol Neurobiol 1997; 17: 245-258.