PhD theses

THE ROLE OF THE OVARIAN STEROIDS IN THE REGULATION OF THE HYPOHYSEAL TROPE-HORMONES

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INTRODUCTION

The function of the endocrine glands and their target organs is regulated by the hypothalamus. The releasing and inhibiting hormones produced by the parvocellular cells of the hypothalamus reach the trop-hormone producing cells of the anterior pituitary via the portal circulation. The sexual steroids influence the hormone production of the anterior pituitary partly acting through the hypothalamus, partly through the receptors in the anterior pituitary. It is the well-known feedback mechanism.

Nowadays more and more teenagers choose oral contraceptive to prevent an unwanted pregnancy. Physical development of young people today is faster, so they reach the sexual maturation earlier than the previous generation. On the basis of a Hungarian study half of the fifteen-year-old children are estimated to have been over with the first sexual experience, and the ratio of the unwanted pregnancies is higher between the adolescent compared to adult women. Today pubertal contraception is essential. Contraceptive pills are prescribed to relief menstrual problems and to treat different forms of acne of pubertal girls and young women. To treat these problems in the clinical practice sexual steroids are used for months or for years.

Enviromental estrogens pose a health risk to humans. Drinking water contaminated by metabolites of steroid containing medicine or drinking milk of contaminated by hormones have estrogen like effects and they cause adverse changes in the development and function of male reproductive organs.

AIM OF EXPERIMENTS

In our experiments we tried to imitate the chronic treatments likewise in clinical practices. Female and male rats were used for our experiments. Silastic capsules containing diethylstilbestrol (DES), progesterone (P) or their combinations were implanted under their skin of the neck for two or five months.

We aimed to study whether:

I. two or five month long DES and P treatment administered by *in vivo* how can influence:

1. the opening of the vaginal membrane and the vaginal smears in female rats,
2. the body weight and body length,
3. the weight of anterior pituitary, ovaries, testes and seminal vesicles,
4. the distribution of classical trop hormones, VIP, PACAP and S-100 immunoreactive folliculostellate cells in the anterior pituitary, and
5. the basal plasma hormon levels in both sexes.

II. It was also investigated whether P is able to influence the changes caused by DES in the above-mentioned parameters.

III. Finally we studied that, after removing DES capsule, the above-mentioned parameters are able to return to the control levels.

MATERIALS AND METHODS

Experiental animals

Twenty-five day old Sprague-Dawley female and male rats were used for our experiments.

Experimental groups

We formed the following experimental groups from both sexes:

Experiment I:

1. DES implanted rats
2. DES+P implanted rats
3. P implanted rats
4. Empty capsule implanted rats (empty capsules sealed by Szilorfix silicon based glue were implanted for these animals)
5. Age matched control rats.

Two months after implantation the animals were decapitated.

Experiment II:

In this experiment groups similar to the experiment I were formed. These animals were left alive for five months.

Experiment III:

Two months after DES implantation the capsules were removed. Half of the animals was left to survive further one month, while the other half were left stay alive for further two months before being decapitated. Both experimental groups have age-matched controls too.

Experimental protocol

Silastic capsule containing DES, P or their combination were implanted under the skin of the neck of the animals. Opening of the vaginal membrane of treated and untreated females were recorded. After opening of the vaginal membrane vaginal smear was taken daily for two weeks and two weeks before sacrificing.

At the end of the experimental period the body weight and body length were measured and the animals were decapitated between 9 am and 11 am.

Methods

After decapitation trunk blood was collected to determine the hormone (LH, FSH, PRL, GH) levels by radioimmunassay (RIA). The anterior pituitary, ovaries, testes and seminal vesicles were removed and weighted.

D*irect RIA of LH, FSH, PRL and GH*

Plasma was used to determine the basal hormone levels. The trunk blood was centrifugated at 4°C with 1500 r 20 min. Plasma was stored at -20°C until use. LH, FSH, PRL antiserum raised in rabbit or GH antiserum raised in monkey was added to the iodinated samples. Hormone levels were determined in ng/ml plasma. The mean of two parallel determinations for each animal was subjected to Student's *t*-test. $P < 0.05$ was considered significant.

Immunohistochemistry

Three animals of each group were perfused with paraformaldehyde (PFA). The pituitaries were removed and postfixed overnight. The pituitaries PFA fixed or immersed in Bouin solution were washed in 0.1 M potassium-phosphate buffer (KPB) and they were put in ascending sucrose solution (10- 20-30%) for cryoprotection. One day later they were embedded in cryomatrix and then 20 μm thick sections were cut on cryostate in 8 parallel series. Some sections were used for hematoxylin-eosin staining to demonstrate the presence of prolactinomas, and the other sections were stained for trop-hormones immunoreactivities.

RESULTS

EFFECT OF STEROID TREATMENTS ON THE OPENING OF THE VAGINAL MEMBRANE AND THE VAGINAL SMEARS

Opening of the vaginal membrane: In intact rats the vaginal membrane opened on 31th day of postnatal life. Upon DES treatment the opening of the vaginal membrane was advanced with three days. DES+P treatment did not prevent the effect of DES. P alone had no effect.

Vaginal smears: Upon DES treatment persistent estrus was observed. DES+P or P alone did not interrupt the cyclicity, but it was irregular and metestrus predominated. After the removal of DES capsule the cycle gradually returned to normal.

EFFECT OF STEROID TREATMENTS ON THE BODY LENGTH AND BODY WEIGHT IN DIFFERENT EXPERIMENTAL GROUPS

Body length: DES and DES+P administration for two months diminished the body length and body and tail length significantly in both sexes compared to the controls. Upon five month-long DES+P treatment the P moderated the effect of DES, in females the body length reached the control level, but in males it remained under the control value. After removal of DES the body length returned to the control level in females but in males it remained lower.

Body weight: The changes in body weight were similar to those in the body length of both sexes in two month DES and DES+P treated groups; in five month treated groups P blunted the effect of DES in both sexes. Five-month treatment with P alone mildly decreased the body weight in males. After the removal of DES the body weight did not return to control level in either gender.

EFFECT OF STEROID TREATMENTS ON THE WEIGHT OF ORGANS IN DIFFERENT EXPERIMENTAL GROUPS

Weight of the anterior pituitary: The weight of the anterior pituitary increased upon DES, DES+P treatments in the case of two month survival in both sexes. After administrating five month-long combined treatment the P reversed the increased anterior pituitary value. One month after removing DES the weight of the anterior pituitary returned to the control level in males and females.

Weight of ovaries: Treating the female rats with DES and DES+P for two months, we observed reduced weights. Upon five month-long combined treatment the P prevented the effect of DES. P alone slightly reduced the weights only upon five-month-long treatment. One month after DES removal the weight of ovaries was similar to that of controls.

Weight of testes and seminal vesicles: DES and DES+P administrations for two months decreased significantly the weight of testes and seminal vesicles. During combined treatment P could not prevent the intense loss of weight for these organs. In the case of five-month-long treatment DES reduced the weight of these organs to a great extent, while P given in combination with DES was able to blunt the effect of DES. P alone had no effect. Two months after DES removal we observed significantly lower weight of testes compared to the controls, while the reduced weight of seminal vesicles gradually returned to the control values.

EFFECT OF STEROID TREATMENTS ON THE BASAL PLASMA LEVELS MEASURED BY RADIOIMMUNOASSAY (RIA)

The basal LH plasma level: The different treatments did not result any changes in the basal LH level in females. In males both DES and combined treatments reduced significantly the basal LH

level, which returned to the normal value after five months in DES treated animals, but not in DES+P treated ones. P alone had no significant effect. One month after removing DES the basal LH levels remained low, while two months after DES removal it returned to the control level.

The basal FSH level: In females we could not observe any changes in the basal FSH level upon two or five month steroid treatments. In males both DES and DES+P administered for two months diminished the basal FSH level, but after five months the values were similar in each male groups. P alone had no significant effect. After removing DES the basal FSH level remained low in males.

The basal PRL level: DES drastically increased the basal PRL level, during two-month-long combined treatment the increase was less significant, in the case of five month survival the P was able to prevent the effect of DES on the PRL level in both sexes. P alone had no effect. After DES removal the PRL level gradually returned to the control level.

The basal GH level: The observed changes of the GH level were controversial. The basal GH level was very low in both sexes. In females only the two-month-long, but not five-month-long, DES treatment caused significant decrease in the GH level, and in males only the two-month-long DES+P treatment elevated the GH level. In females one month after removing DES the GH level was similar to that of control level.

EFFECT OF STEROID TREATMENTS ON THE DISTRIBUTION OF THE CLASSICAL TROP-HORMON PRODUCING CELLS IN THE ANTERIOR PITUITARY

The results of immunohistochemistry well correlated with the results of RIA.

Distribution of LH and FSH immunoreactive (ir) cells: The LH and FSH ir cells are round or oval-shaped in both males and females, the largest number of LH and FSH ir cells can be seen in the gonadotropic zone of the anterior pituitary, within the other portion of the gland where they are evenly distributed. The effect of different treatments on the LH and FSH immunoreactivities was similar in both sexes. Upon two-month-long DES treatment the number of LH and FSH ir cells was reduced. After five-month-long DES administration we could observe more intense effect. During combined treatment the P prevented partially the effect of DES, but the density of LH and FSH cells in gonadotropic zone was lower than that in controls. P alone had no effect. Already one month after DES removal the number and distribution of gonadotropes were similar to that of controls in both sexes.

Distribution of PRL ir cells: Prolactin cells are evenly distributed within the anterior lobe of both males and females. The PRL producing cells are small, and cup-shaped due to the accumulation of immunoreactive material at only certain part of these cells. Steroid treatment caused similar changes on PRL immunoreactivity in both males and females. Two months after DES implantation the size and the number were enlarged compared to the controls. They had round or oval shape, and their diameter

doubled compared to the diameter of the normal cells in the anterior pituitary. DES treatment of five months the PRL cells proliferated and hypertrophyzed and they formed small or large prolactinomas. During two or five-month combined treatments, the size of PRL cells decreased and they did not form prolactinomas, they were not densily packed, but they lost their cup-shape, as the ir material filled in the entire cells. P alone had no effect. The changes in the number and distribution of PRL cells regressed already one month after DES removal.

Distribution of GH ir cells: The GH producing cells are small and round, in intact rats they are evenly distributed, and they are missing from the gonadotropic zone in the anterior pituitary. There is no difference in their immunohisthochemical appearence in both males and females. We could not observe any changes in the distribution of GH ir cells after two-month-long DES treatment; however, small empty areas were detected indicating the location of the developing prolactinomas. DES treatment of five-months led to a considereble change in the distribution of these cells. PRL and GH double labeling revealed that the adenomas formed by PRL cells did not contain any GH cells, but these cells could be observed at the periphery of the prolactinomas. Upon DES+P treatment the histological appearance was similar to that of controls; however GH cells could be detected in the gonadotropic zone;however, this zone only containes gonadotrope and PRL cells in intact rats. Two months after the removal of DES capsule the histological appearance was similar to that of controls.

Distribution of VIP ir cells: As it is known in intact rats these cells are missing or only few can be found in the anterior pituitary. Applying steroid treatments in both sexes the changes were similar. Increased number of VIP ir cells were detected two months after DES administration in the anterior pituitary. Upon five-month-long treatment with DES not only the number but the size of VIP cells also increased significantly. The enlarged VIP cells formed VIP-omas. Applying combined treatment P was able to prevent the occurrance of VIP-omas, and evenly distributed VIP cells were seen in the anterior pituitary. P alone had no effect. One month after removal of DES the number of VIP cells decreased and the histological appearance was similar to that of control two months after DES removal.

Distribution of PACAP ir cells: PACAP ir cells were only observed in proestrus female rats. In other stage of estrous cycle of females and in males PACAP ir cells were not present. Steroid treatment did not induce appearance of PACAP ir cells in these latter groups.

Distribution of folliculostellate cells: S-100 immunoreactivity is a marker for folliculostellate cells. These stellate-shaped cells have long processes embracing the tropic-hormone producing cells. They are always present in intact animals, they distribute evenly in the anterior pituitary, and form a well-defined border between the anterior and intermediate lobes. Two-month-long DES treatment did not make any significant changes in the distribution of S-100 ir cells. After five-month-long DES treatment these cells concentrically surrounded the prolactinomas forming demarcation around them. PRL and S-100 double labeling revealed that there were only a few S-100 ir cells in prolactinomas. In

DES+P and P treated rats the distribution of S-100 cells did not change. After removing DES the distribution of these cells was similar to that of controls.

DISCUSSION

The majority of the data found in the literature are *in vivo* or *in vitro* observations. In the clinical practice chronic hormone treatments are used which have numerous side effects. With regard to these circumstanses we investigated the effects of long-term (two- or five-month-long) gonadal steroid administration on the hypophyseal hormone secretion, with special attention to sexual differences in both sexes and we also studied how P could influence the undesirable effects provoked by long-term E treatment. On the other hand we studied whether the effects provoked by two-month-long DES administration were reversible after removing of DES capsule.

Effects of treatments on the PRL secretion

The observed changes of the PRL secretion were similar in both sexes. The PRL cells hypertrophyzed, and after five-month-long E treatment prolactinomas developed, which were probably responsible for the increased weight of the anterior pituitary. Most S-100 ir cells could be observed at the periphery of prolactinomas forming a border around them, and within them only a few S-100 ir cells were detected. The special arrangement of S-100 cells around the prolactinomas and their known role in the lacotorop hypertrophy and hyperplasia suggest that PRL cell proliferation is not interstitial but appositional. The prolactinomas contained a lot of dilated vessels. The number of VIP ir cells parallel to the PRL cell hypertrophy increased and they formed VIP-omas during five-month-long DES treatment.

The S-100 and VIP cells play important role in the PRL cell hypertrophy. Results obtained by RIA show that E stimulates the VIP content in the anterior pituitary (Rosténe, 1984). Application of VIP antibody reduced the PRL secretion in the anterior pituitary (Hagen et al, 1986), and the number of VIP ir cells in anterior pituitary cell cultures (Carretero et al, 2006). Nagy and his co-workers (1988) using haemolytic plaque assay demonstrated that the PRL release was stimulated by VIP in an autocrine manner. In our previous experiments we found colocalization between PRL and VIP cells. These results confirm the hypothesis VIP is able to influence the PRL secretion in an autocrin manner (Köves et al, 1990; 1996).

In our present experiments P prevented the increased number of VIP cells caused by DES suggesting that P could prevent lactotrope hypertrophy in this way. The question arises where the target of P is found and how P can prevent the increase of VIP cells. Calderon and his co-workers published in 1987 that E induced the nuclear accumulation of ERs and the occurrance of PRs in the anterior pituitary, at the same time E did not influence these parameters in the hypothalamus. According to our unpublished data, DES treatment significantly increased the density of ERα in the

anterior pituitary and P could prevent this effect. Of course, the effect via hypothalamus can not be also excluded. In D2 knock-out mice the VEGF protein and its mRNA increased suggesting that DA can inhibit the VEGF expression in intact animals. If steroid treatments inhibit DA release it leads not only to elevated PRL level but to increased VEGF content too (Cristina et al, 2005). The portal vessels in intact rats supply the anterior pituitary. E induces not only tumorgenesis, but development of new arteries as well. These new developed arteries do not belong to the hypophyseal portal system, they transport systemic blood, from which DA is missing. The lack of DA inhibition further increases tumor formation.

Two-month-long DES administration did not cause irreversible changes either in the weight of pituitary or in the PRL secretion. One month after removal of DES the weight of pituitary already returned to the control level, while PRL reached the control level by the end of second months.

Effects of treatments on the LH and FSH secretion

In the changes of LH and FSH secretion a charecteristic sexual dimorphism can be observed. DES and DES+P treatment diminished the plasma basal LH and FSH levels only in male rats demonstrating that only the males responded to steroids. P was not able to prevent the effects upon two-month treatment; however, after five months it prevented the decrease of FSH level and blunted the reduced weight of testes. The FSH secretion and the weight of the testes underwent irreversible demage, because after DES removal these parameters did not return to control level, however, the LH level and the weight of seminal vesicles reached the control value. In females only the weight of ovaries decreased because of the complete or partial lack of ovulation, but there was no change in the LH and FSH levels. The weight of ovaries normalized after removal of DES capsule.

Generally there is a sexual dimorphism in the LH and FSH release. In females the LH and FSH are released in a cyclic manner leading to ovulation; however, in males this cyclicity is missing. The above-mentioned sexual differences explain the divergent responses to steroids.

The neural LH release apparatus was firstly described by Everett and Sawyer (1949; 1950). Later it became evident that the GnRH producing cells can be found in the medial septal and preoptic areas in rats. This hormone influences the pulsatile and cyclic secretion of gonadotropic hormone producing cells in the anterior pituitary. E influences the function of the GnRH producing cells in the preoptic area of female rodents and in the medial basal hypothalamus of other mammals acting through positive feed-back and stimulates the GnRH and LH release leading to ovulation (Müller and Nistico, 1989). In males the GnRH is released in a pulsatile fashion, there is no LH-surge, so the positive feed-back of E is lacking (sexual dimorphism), and in the male acyclic hypothalamus there is no E-sensitive GnRH pulse generator (Bourguignon et al, 1993). Sexual difference could be observed in the GnRH and LH response to steroid treatments in prepubertal female rats. In the prepubertal females treated with E the P caused a sigificant change in the GnRH concentration of preoptic and suprachiasmatic areas, which could not be observed in males (Dluzen and Ramirez, 1980). Sexual

dimorphism was described in the response of gonadotropic hormone α-subunit promoter activity to GnRH (Colin et al, 1996).

Shukuwa and his co-workers (2006) investigated the effect of different amount of DES on LH, FSH and PRL cells, on the distribution of ERα and ERβ in anterior pituitary of male mice by immunohistochemistry. DES was administered for twenty days. DES reduced the number of LH and FSH cells, while increased the number of PRL cells in a dose-dependent manner. Upon DES treatment ERβ was observed on lactotropes, but this effect was not determined in ERα knock-out mice. Probably DES acts directly via ERα. The occurrance of Pit-1 gene was also investigated in PRL cells during DES treatment. DES treatment increased the number of Pit-1 positive cells, on the other hand some LH and FSH cells were Pit-1 positive, and these cells colocalized with PRL cells. On the basis of their findings it seems that DES stimulates not only the PRL cell proliferation but the FSH and LH trans-differentiation as well.

How can we explain that the weight of testes did not, but the weight of seminal vesicle returned to the control level after DES removal? The LH level was similar to that of controls after removing DES in males. LH is responsible for the testosteron production and for maintenance of the seminal vesicle function. After DES removal the FSH level remained under the control level. FSH is responsible for the spermatogenesis; this fact explains the reduced weight of testes. Jehan and his co-workers (1971) treated adult rats with rising dose of intramuscular E2 or E2+P combination. Both treatments diminished the weight of testes, and demaged the spermatogenesis and the testosterone production. After the treatments the above-mentioned parameters partly recovered. Amador and his co-workers (1989) treated Fischer 344 rats with DES containing or empty capsule. DES capsule were removed from the half of the treated animals seven weeks after implantation. DES decreased the body weight, the weight of testes and seminal vesicle. During DES administration the number of testicular LH receptors increased, at the same time the plasma gonadotropin level reduced, the PRL plasma level elevated. After DES removal the weight of organs was similar to that of controls. Toyama and his co-workers (2001) investigated the effect of neonatally administered DES on development of the blood-testis barrier in rats. Newborn rats were treated with 10 μg DES from the 2nd to the 12th postnatal days and the testes were sequentially examined up to 105 days of age by light, electron, and confocal microscopy. They observed that neonatal administration of DES delayed the establishment of the blood-testis barrier for four weeks. Consequently, during this period, pachytene spermatocytes were exfoliated from the seminiferous epithelium without completion of meiosis. The experiment suggests that before the puberty steroid enviroment influences not only the development of the blood-testis barrier but the maturation of spermatocytes as well.

On the basis of the above-mentioned data it is possible that in our experiments because of the continuous E influence the blood-testis barrier did not develop leading to an irreversible damage in the testes.

E likely acts directly on the testes. Both ERα and ERβ were described in testes. The role of GH and PRL is not excluded. The testes expresses GH and PRL receptors. GH stimulates not only the development of body cells, but it influences the testosteron production of Leydig cells and the spermatogenesis as well (Hull and Harvey, 2000). The high PRL level reduces the testosteron production. In our experiments DES led to hyperprolactineamia and reduced weight of testes.

Drinking water contaminated by metabolites of contraceptives carries a risk to enviromental pollution. Pregnant mothers were treated by ethynil estradiol. In the male offsprings of adult age the weight of testes and seminal vesicles reduced and the number of spermatozoa decreased in epididymis compared to the controls (Howdeshell and his co-workers, 2008).

Effects of treatments on the body length, body weight and GH secretion

Two month-long DES and DES+P administrations significantly diminished the body length in both males and females. Upon combined treatment applied for five months the P could only revent the effect of DES on the body length of females. P alone had no effect on the body length in both sexes. In females the body length returned to the control value after DES removal; however, it remained low in males. Although two-month-long DES and DES+P treatments reduced the body weight in both sexes, upon five month-long combined treatment the P blunted the DES effect in both sexes. P alone had no effect in females but mildly decreased this parameter in males. After DES removal the decreased body weight did not reach the control level in either sexes.

Data in the literature support the fact that E has impact on the fusion of epiphyseal plate. Nilsson and his co-workers (2003) described that the epiphyseal plates express ER, through which E can influence their fusion. We supposed that in our experiments, upon long-term treatment DES reduced the body acting probably via the epiphyseal plates. The role of E in the fusion of epiphyseal plates are supported by two genetic diseases. One of them is the mutation of the aromatase enzym coding gene (Morishima et al, 1995), the other is the mutation of the ERα coding gene. Both cause the insufficiency of epiphyseal plate's fusion (Smith et al, 1994). Within the epiphyseal plate Nilsson and his co-workers (2002) distinquised three zones (reserve, proliferative, degenerative). They investigated the expression of ERα and ERβ in these zones of rats and rabbits at different postnatal age. The plates in rabbits and in rats express both receptor types; however, their distribution is different depending on the age. The reserve and proliferative zones express ERα and ERβ in both species, while these receptors were present in a few number in the degenerative zone at early postnatal age, and then their number increased till the fusion of epiphyseal plate. Vidal and his co-workers (2000) studied the longitudinal bone growth in ERα or ERβ knock-out mice. Reduced bone growth could be detected only in the ERα knock-out mice. According to these observations E acts via ERα receptors on the epiphyseal plate. McMillan and his co-workers (2006) hypothesized that E could modulate the function of the epiphyseal plate via a sex-specific MAPK pathway. This pathway was detected only in females. In the last decades a lot of enzymes have been described in the proliferative zone of the

chondrocytes participating E2 production. Chagin and his co-workers (2006) measured the E2 production of human chondrocyte cultures by RIA. They observed that these cells produced E2 of significant amount. Treatment with aromatase inhibitor and ER antagonist inhibited the chondrocytes proliferation and stimulated their apoptosis. It seems that the locally produced E has proliferative and apoptotic effects.

Romano and his co-workers (2003) described that both PACAP38 and VIP activate the Rap-1 via VPAC2 receptor, which increase the PRL gene transcription in lacto-somatotropes. PAC1, VPAC1 and VPAC2 receptors were detected in transplantable hypophyseal tumors expressing PRL and GH (Vertongen et al; 1996). VIP affects PRL secretion through VPAC receptors.

In our experiments DES administration decreased the body weight in both sexes. Presumably the reduced GH level and the increased PRL level are responsible for the loss of body weight. GH receptor can be found in the liver, in the adipose tissue, in the pancreas (Billestrup és Martin, 1985; Nielsen éet al, 1990). Similar to the GH receptors PRL receptors were also detected in the liver and in the pancreas. GH increases the protein synthesis, carbohydrate and fat matabolism. The role of PRL in the regulation of body weight is not clear, some authors described increase, while the others reported decrease (Ben Jonathan et al, 2006) in the body weight upon PRL influence.

The question arises how E can display its effect, and how P can influence the effects provoked by E during the treatments. Both E and P act through the above-mentioned specific receptors. E and P receptors were detected in the brain and in the pituitary, so the sexual steroids can exert their effect on the hypotalamus or directly on the pituitary. Gonzales and his co-workers (2008) described ERα receptors in the high number on the lactotropes, somatotropes, thyreotopes, and on the gonadotropes in low number. ERβ is present only in a few number in the anterior pituitary, which were expressed by the somatotropes, lactotropes and gonadotropes. PR-A immunreactivity was seen in the nucleus of gonadotropes (Garrido-Gracia et al, 2007). The distribution of both ER types in the hypothalamus was thoroughly mapped by Merchenthaler and his co-workers (2004). The ARC and the anterior PV areas expressed ERα in the greatest number, while ERβ was found only in the PVN and in the preoptic area. In these nucleus ERβ immunoreactivity colocalized with the GnRH (Hrabovszky et al, 2001). Neurons containing PR were detected in the ventromedial nucleus and medial portion of ARC (Leranth et al, 1992). Calderon and his co-workers (1987) investigated the effect of P in the hypothalamus and in the anterior pituitary of ovariectomized, immature E treated female rats. Upon E administration the nuclear accumulation of ER increased and the occurrence of PR reached its surge after 12 hours, then its number continously decreased, finally it returned to the control level. When the animals were treated with P the nuclear accumulation of ER induced by E decreased during the PR surge. These changes could be seen only in the anterior pituitary, not in the hypothalamus. According to these results P influences the responsiveness of anterior pituitary to E. It seems that there could be a cross-talk between the ER and PR at the pituitary level.

Taking these results into consideration, there are some ways how to explain the protective effect of P. 1). PR-A is present in gonadotropes. P binding to gonadotropes influences the E binding to lactotropes and in this way the production of TGF-β3 may reduce. 2). ERs in lactotropes may have P responsive elements and P can bind directly to these receptors to prevent production of TGF-β3. 3). The effect of P through the hypothalamus is not excluded. PRs were detected in the hypothalamic nuclei suggesting P can act not only through the pituitary but via the hypothalamus as well.

SUMMARY OF THE NEW RESULTS

We have received the following results. **1**. Upon steroid treatments the opening of the vaginal membrane was significantly advanced. DES resulted in persistent estrus. DES+P and P did not interrupt the cyclicity but it was irregular and metestrus predominated. **2.** DES treatment diminished the body weight, body length in both sexes. In five months P attenuated the effect of DES on the body length and body weight of females and the body weight of males. **3.** DES enhanced the weight of the anterior pituitaries and diminished the weight of gonads and seminal vesicals. P blunted the effect of DES on the weight of the above-mentioned organs. Two months after the removal of DES capsule the weight of anterior pituitaries, ovaries and seminal vesicals returned to the control level; however, the weight of testes gradually neared to, but not reached the control level. **4.** Except of PRL there was a sexual dimorfism in the changes of tropic hormone levels upon the treatments. The effect of DES treatments on PRL levels was the same in both genders, the basal PRL levels extremly enhanced and prolactinomas developed, it was accompanied by the enhancement in the weight of anterior pituitaries. P in the case of two-month treatment attenuated, and in the case of five-month treatment prevented the effect of DES. The changes was reversible. After the removal of DES capsule the PRL levels and the weight of anterior pituitaries returned to control level. The changes in the basal LH and FSH levels showed sexual dimorfism. Basal LH and FSH levels declined only in male rats upon DES and DES+P treatment. After the removal of DES capsule the FSH levels and the weight of testes remained low. It is possible that in our experiments because of the continuous E influence the blood-testis barrier did not develop leading to an irreversible damage in the testes; however, the LH levels and the weight of seminal vesicles normalized by the end of the second month. The reduced weight of ovaries caused by DES treatment also normalized because after the removal of DES the ovulation returned and the corpora lutea appeared. There was a mild sexual dimorfism in the GH levels. In the case of two-month treatment DES depressed the GH level in females but did not influenced it in males; however, the body length and body weight were lower in both male and females than in their control groups. In the case of five-month DES+P treatment P could partially attenuate the loss of body weight of of both sexes and prevented the decrease in body length of females. In the case of the removal of DES the body weight remained lower in males than in the control group, in females it came nearer the control level. **5.** We have found correlation between the immunohistochemistry and the basal plasma hormone

levels. DES extremly depressed the number of LH and FSH cells, although the number of PRL cells enhanced, in the case of five-month survival prolactinomas developed. GH cells were evenly distributed but they were not present in the prolactinomas. P prevented the above-mentioned changes. Two months after the removal of DES capsule the immunohistochemical appearance of LH, FSH, PRL and GH cells were similar to that of the control animals. **6**. The number of VIP immunoreactive cells enhanced upon DES treatment in both sexes. In the case of five-month survival VIP-omas developed. P prevented the effect of DES. **7.** In both sexes the different treatments did not cause the occurrance of PACAP immunoreactive cells in the anterior pituitary. **8**. The S-100 immunoreactive folliculostellate cells embraced the prolactinomas, but inside them they rarely appeared.

CONCLUSION

It was concluded that there was a sexual dimorfism in the effect of steroid treatments on the basal plasma LH and FSH levels, the body length and body weight, and the weight of gonads and seminal vesicles; however, the steroid treatments similarly influenced the PRL levels, the weight of pituitaries, the number and distribution of VIP and folliculostellate cells in both sexes. It did not induce the appearance of PACAP ir cells.

On the bases of our unpublished data and those available in the literature we suppose that DES and P affects the pituitary hormone secretion through specific receptors present in the anterior pituitary itself.

PUBLICATIONS RELATED TO THE THESIS

1. **Andrea Heinzlmann**, Katalin Köves. The charateristic change in the distribution of S-100 immunoreactive folliculostellate cells in rat anterior pituitary upon long-term estrogen treatment is prevented by progesterone. Endocrine, 2008, 33:342-8 (IF:1,842).

2. **Andrea Heinzlmann,** Kirilly, E., Meltzer, K., Szabó, E., Baba, A., Hashimoto, H., Köves, K. PACAP is transiently expressed in anterior pituitary gland of rats. In situ hybridization and cell immunoblot assay studies. Peptides, 2008, 29:571-577 (IF 2.701).

3. **Andrea Heinzlmann**, Katalin Köves, György M. Nagy. Effect of concomitant progesterone administration or the effect of removal of estrogen capsule on changes caused by long-tem estrogen treatment in pituitary VIP immunoreactivties. Endocrine, 2010, 37:396-402 (IF:1,842).

4. **Andrea Heinzlmann**, Katalin Köves, Magdolna Kovács, Valér Csernus. Sexual dimorphism in the effect of concomitant progesterone administration on changes caused by long-term (two or five months) estrogen treatment in pituitary hormone immunoreactivities of rats. Med Sci Mon, 2010, 16 in press (IF: 1,543).

PUBLICATIONS NOT RELATED TO THE THESIS

K. Köves, G. Kiss, **A. Heinzlmann,** J. Takács, R. Dochnal, M. Mancinger, Á. Pál, I. Sípos, G. Szabó. Secretin attenuates the hereditary repetitive movements in a mouse model. Journal of Molecular Neurosci, 2010, (DOI 10:1007/s12031-010-9408-2)

A. Heinzlmann, Zs. Tóth, K. Köves. Secretin mRNA in the Subdivisions of Primary Sensory Neurons in the Trigeminal Ganglion of Rats. Journal of Molecular Neurosci, 2010, (DOI 10.1007/s12031-010-9395-09-0)

K. Köves, M. Kausz, D. Reser, Gy. Illyés, J. Takács, **A. Heinzlmann**, E. Gyenge, K. Horváth: Secretin and autism: a basic morphological study about the distribution of secretin in the nervous system, Regulatory Peptides, 2004, 123:209 –216 (IF: 2,235).

K. Köves, O. Kántor, J Molnár, **A. Heinzlmann,** E. Szabó, F. Szabó, Á. Nemeskéri, J. Horváth, A. Arimura. The role of PACAP in Gonadotropic hormone secretion at hypothalamic and pituitary levels. J.Mol.Neurosci, 2003, 20:141-152 (IF:1,735).

F. Szabó, J. Horváth, **A. Heinzlmann**, A. Arimura, K. Köves: Neonatal PACAP administration in rats delays puberty through the influence of the LHRH neuronal system. Regulatory Peptides, 2002, 109:49-55 (IF:3,205).

O. Kántor, **A. Heinzlmann**, N. Suzuki, E. Vincze, K. Köves: Distribution of PACAP and its mRNA in several nonneuronal tissues of rats demonstrated by sandwich enzyme immuassay and RT-PCR technique, Regulatory Peptides, 2002, 109:103-105 (IF:3,205).

E. Szabó, Á. Nemeskéri, **A. Heinzlmann**, N. Suzuki, A. Arimura, K. Köves: Cell immunoblot assay study demonstrating the release of PACAP from individual anterior pituitarity cells of rats and the effect of PACAP on LH release. Regulatory Peptides, 2002, 109:75-81 (IF:3,205).

K. Köves V. Verecki,, M. Kausz, O. Kántor, J. Molnár, Á. Nemeskéri, **A. Heinzlmann**, E. Szabó, F. Szabó, K. Fógel, A. Lakatos, G. Szeiffert, A. Arimura: PACAP and VIP in photoneuroendocrine system (PNES). Medical Science Monitor 2002, 8:SR5-20 (IF:1,381).

O. Kántor, J. Molnár, **A. Heinzlmann**, A. Arimura, Zs. Fürst, K. Köves: Study on the hypothalamic factors mediating the inhibitory effect of PACAP38 on ovulation, Peptides, 2001, 22: 2163-2168 (IF:2,092).

O. Kántor, J. Molnár, **A. Heinzelmann**, Z. Fürst, A. Arimura, K. Köves: The inhibitory effect of PACAP38 on the ovulation is mediated by CRF and endogenous opioid. Ann. NY, Acad. Sci, 2000, 921: 405-409 (IF:1,381).

ORAL PRESENTATIONS AND POSTER

2009. The 9th International Symposium on VIP, PACAP and Releated Peptides, Kagoshima, Japan K.Köves, G. Kiss, M. Mácsai, **A. Heinzlmann**, Á. Csáki, J. Takács, R. Dochnal, Z. Boldogkői, G. Szabó: Secretin attenuates the repetitive hyperactive movements in a mouse model. (oral presentation)

2009. The 9th International Symposium on VIP, PACAP and Releated Peptides, Kagoshima, Japan

A. Heinzlmann, Katalin Köves: Secretin expressing primary sensory neurons in the trigeminal ganglion of rats: *in situ* hybridization study (oral presentation)

2008. Annual Meeting of Postgraduate Students, Budapest, Semmelweis University, Hungary

A. Heinzlmann., K., Köves.: Progesterone combined by oestrogen fends off the changed distribution of S-100 immunoreactive cells caused by oestrogen (oral presentation)

2007. XIX. International Symposium of Morphological Sciences, Budapest, Hungary

A. Heinzlmann., M. Kovács., K. Köves.: Undesired changes caused by Long-Term Estrogen Treatment in the Immunoreactivity of LH, FSH, PRL, ACTH and S-100 (present in folliculostellate cells) is Modified by Concomitant Progesterone Administration (oral presentation)

2006. Annual Science Day, Budapest, Hungary

A. Heinzlmann., K., Köves.: Is there a difference in the hormone secretion of the anterior pituitary during a long-term oestrogen and combined oestrogen-progesteron treatment as to sexes? (oral presentation)

2005. 7th International Symposium on VIP, PACAP and Related Peptides, Rouan, France

A. Heinzlmann., K., Köves., Estrogen induced in vivo proliferation of VIP and prolactin immunoreactive cells in the anterior pituitary is moderated by progesterone (poster).

2005. 13th. Congress of the Society of Hungarian Anatomist [MAT] , Pécs, Hungary

A. Heinzlmann., K., Köves.: Progesterone is partially able to blunt the undesirable effects of long-term oestrogen treatment in the anterior pituitary. (poster)

2005. Annual Meeting of Postgraduate Students, Budapest, Semmelweis University, Hungary

A. Heinzlmann., K., Köves.: Role of the ovarial steroids in the regulation of the VIP and gonadotropic hormone secretion of the anterior pituitary. (poster)

2002. 18th Congress of Hungarian Endocrinologist and Anatomist [MEAT], Lillafüred, Hungary

Kántor. O, Molnár J, **Heinzlmann A,** Fürst Zs, Arimura A, Köves K: Effects of the gastrointestinal peptides on the regulation of the ovulation in cyclic female rats.

2002. 19th Congress of Sociaty of Hungarian Endocrinologist and Anatomist, [MEAT], Gyula, Hungary

Szabó F, **Heinzlmann A,** Horváth J, Arimura A, Köves K:. Neonatally treating with PACAP defers beginning of puberty through LHRH neuronal system (oral presentation)

2002. Annual Meeting of Students~ Researchers (TDK), Budapest, Semmelweis University

Szabó F. ÁOK VI., **Heinzlmann A.**:. The deferred effect of PACAP on puberty exerts via LHRH neuronal system. (oral presentation)

2002. 19th Congress of Hungarian Endocrinologist and Anatomist, [MEAT], Gyula, Hungary

Heinzlmann A., Kántor O., Suzuki N., Kocsis K: Distribution of PACAP and its mRNA studied by sandwich enzim immunassay and RT-PCR technique. (oral presentation)

2001. 5th. International Meeting: VIP, PACAP, Secretin, Glucagon and Related Peptides, Santa Barbara, USA

O: Kántor, **A. Heinzlmann**, N. Suzuki, E. Vincze, K. Kocsis, K. Köves:

Distribution of PACAP and its mRNS in non-neuronal tissues of rats demonstrated by sandwich enzyme immunassay and RT-PCR technique (poster)

2001. Annual Meeting of Students Researchers (TDK), Budapest, Semmelweis University

Heinzlmann A.: CRF and endogenous opioids mediate the inhibiting effect of PACAP on ovulation. This oral presentation was awarded with a rectorial prize

2001. Annual lecturing course organized by Frigyes Korányi Tradecollege

Heinzlmann A.: CRF and endogenous opioids mediate the inhibiting effect of PACAP on ovulation. (oral presentation)

PUBLICATIONS IN BOOKS

Köves, K., **Heinzlmann, A.**,: The role of PACAP in biological rhythms. In: Neuropeptides and Peptide Analogs, Research Signpost, New York, 2008, pp. 143-160.

Köves, K., **Heinzlmann, A.:** Neurotransmitters and Neuropeptides in Autism. In: New Autism Research Developments. Ed: Mesmere B., Nova Science Publishers, Inc. New York, 2007, pp. 1-67. (1 chapter)

Köves K. Vereczki V., Molnár J., Kántor O., **Heinzlmann A.,** Szabó E.: Presence and role of pituitary adenylate cyclase activating polypeptide in the photoneuroendocrine system. In: Rhythmic biological processes. The role of biological clocks. Editors: Csernus V., Mess B. Dialóg Campus Kiadó, Budapest-Pécs, 2003, pp. 147-164. (1 chapter)

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