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ABSTRACTS

THE EFFECT OF OXYTOCIN AND VIP ON THE LH SECRETION BY PORCINE PITUITARY CELLS AFTER TREATMENT WITH EXOGENOUS ESTROGEN

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The objective of this study was to determine whether LH secretion by pituitary cells of pigs is modulated by OT and VIP and whether oestradiol benzoate (BE) influence on LH secretion and sensitivity of cells. Pituitaries were removed from ovariectomized gilts. Animals were divided into groups: I-control, II-experimental received BE injection (25 μg/kg b.w.) 30-36 h before slaughter, III-experimental group received of BE at the dose as above 60-66 h before slaughter. The anterior pituitary gland was separated, washed in Dulbecco's medium and finaly minced tissues was digested in 0.3% trypsin. Pituitary cells were diluted to 100,000 cells/ml with McCoy's 5A culture medium. The cells suspension was cultured 48 h at 37°C in 95% air and 5% CO_2. After seeding, plates were rinsed and pituitary cells were cultured 3.5 h in medium containing: 100 ng/ml GnRH, (10^{-6}, 10^{-7}, 10^{-8}M) OT, (10^{-7}, 10^{-8}, 10^{-9}M) VIP and antagonist VIP at dose 10^{-7}M. It was found that the addition of BE 60-66 h before slaughter caused significant ($P<0.01$) increase in LH release into the culture medium in the presence of all doses of OT and VIP as comparised to group without the treatments. In the remaining groups OT and VIP had no effect on LH secretion. The obtained results suggest that treatment with BE 60-66 h before slaughter increases the sensitivity of pituitary cells to OT and VIP and enhances the LH secretion *in vitro*.

CONTROL OF GONADOTROPIN SUBUNITS AND GnRH RECEPTOR GENE EXPRESSION

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The secretion of pituitary gonadotropins is modulated by complex interrelationship between several hormones among which sex steroids and hypothalamic GnRH play major roles. These factors individually regulate the synthesis of gonatotropin subunits α, LHβ and FSHβ at least in part by modulating gene expression. Steroids may operate directly at the pituitary level or indirectly at the hypothalamus to alter pulsatile secretion of GnRH, and they can have positive or negative actions, depending on the experimental model and physiological conditions. Specifically, sustained stimulation by GnRH results paradoxically in desensitization of gonadotrope cells and an inability to maintain increased gonadotropin gene expression, a property which has been exploited for several applications including reproduction and treatment of cancer. GnRH acts *via* a 327-328 amino acid long, seven-transmembrane domain G-protein coupled receptor (GnRHR) encoded by a single gene. The expression of the GnRHR gene appears to be under positive or negative regulation by steroids or GnRH. The mechanisms by which GnRH and steroids modulate gonadotropin and GnRHR genes are still unclear. Presence of responsive elements in the promoter region of genes, especially the α and LHβ and GnRHR genes, is consistent with a direct action of steroids on gonadotrophs. However, suppressive effects appear to occur primarily *via* hypothalamus targetting, resulting in decreased pituitary stimulation by GnRH. GnRH action involves several signaling pathways that have potential to operate in an interconnected manner for regulating GnRHR and gonadotropin subunits synthesis. Together the data demonstrate that both synthesis and release of pituitary gonadotropins is multihormonally regulated, in a manner that involves multiple routes and mechanisms and includes the integrated regulation of a family of participating gonadotroph genes.

EFFECT OF ACTH1-24 ON PITUITARY RESPONSIVENESS TO EXOGENOUS GnRH AND ON OESTRADIOL-INDUCED LH SURGE IN THE EWE

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This study examined the effect of ACTH1-24 on LH secretion. Oestrus was synchronized with progestin sponges and prostaglandin (PG). Twenty four hours after PG, 18 ewes received 50 μg oestradiol (E2) and 8 h later 3 injections of GnRH (500 ng) at 2 h intervals. This regimen was repeated but all ewes were also treated with ACTH1-24 (0.8 mg i.m.) either 7.5 h (Group 1; $n = 9$) or 5.5 h (Group 2; $n = 9$) after E2. The LH responses to the 2nd and 3rd GnRH injections were greater than the first ($P<0.01$). ACTH1-24 did not alter LH secretion in response to any of the GnRH injections in both groups. In control ewes, 16 out of 18 had an LH surge during the sampling period whereas after ACTH1-24 only 3 ewes in Group 1 and 1 ewe in Group 2 had an LH surge. ACTH1-24 significantly increased the plasma cortisol ($P<0.001$) and progesterone ($P<0.05$) concentrations. In conclusion, during the follicular phase in ewes, ACTH1-24 did not reduce pituitary responsiveness to exogenous GnRH but did block oestradiol-induced LH surges by acting at the hypothalamus. This latter effect was probably mediated by progesterone.

THE EFFECT OF MELATONIN ON SUCKLING-INDUCED OXYTOCIN RELEASE IN THE RAT

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The aim of the present investigation was to study the effect of melatonin (MEL) on suckling-induced oxytocin (OT) release as well as to determine whether this effect depends on the dose of MEL used. The experiments were performed on female Wistar rats. At 8-12 days of lactation each animal was separated for 12 h from all but one pups of her litter. On the day of experiment, anesthetized mothers were cannulated intracerebroventricularly (icv) and then injected icv with MEL vehicle (1% ethanol in 0.9% NaCl) or MEL solution (10 pg/ml, 1 ng/ml, 100 ng/ml or 10 μg/ml), respectively. Fifteen minutes later (not less than an hour after the end of icv cannulation) 8-12 pups were allowed to suck for 15 min. Hypothalamic and neurohypophysial OT contents as well as OT plasma concentrations were measured by RIA. Suckling stimulated the release of OT. 1 ng/ml MEL produced a significant inhibition of suckling-induced OT release; the injection of MEL in doses of 100 ng/ml or 10 μg/ml did not modify the OT release brought about by suckling. Thus, MEL (at concentrations within physiological range) seems to inhibit suckling-induced OT release. When applied in higher doses, MEL was shown not to influence the suckling-induced release of OT.

NEUROPEPTIDE-Y AND LHRH RELEASE FROM THE STALK MEDIAN EMINENCE (SME) OF GILTS DURING ESTROGEN-INDUCED LH SURGE

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The purpose of this study was to examine whether Neuropeptide-Y (NPY) may affect LHRH release during the negative and positive feedback induced with estradiol benzoate (EB). Twelve gilts were ovariectomized (OVX) at 7-8 months of age and one month later assigned to one of the following groups: control (untreated; $n = 4$) and EB I (the negative feedback; $n = 4$) or EB II (the positive feedbeck; $n = 4$) slaughtered 30-36 h and 60-66 h after EB injection, respectively. The SME explants were incubated in Krebs-Ringer bicarbonate buffer (KRB) with 1mg ml^{-1} glucose and 2 x 10^{-5} M bacitracin in an atmosphere of 95% O_2 and 5% CO_2 at 37°C in 30 min periods.The SME tissues after preliminary incubation in KRB alone were incubated in the presence of 3.7 x 10^{-6} M NPY. LHRH concentration was determined by RIA. NPY increased LHRH release from SME in control and EB I groups as compared to basal values respectively; 142.1 ± 13pg ml^{-1} vs. 39.7 ± 6pg ml^{-1} and 290.9 ± 9pg ml^{-1} vs. 132.6 ± 26pg ml^{-1} but decreased in EB II group (103.6 ± 19pg ml^{-1} vs. 289.3 ± 19pg ml^{-1}). These results suggest that NPY affects LHRH release from the SME nerve terminals during preovulatory surge of LH in sows.

PUTATIVE INVOLVEMENT OF MELATONIN IN STRESS AND IMMUNOLOGY

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In the past several years it has become increasingly evident that the immune and the neuroendocrine system can be define as the immune-neuroendocrine network. The melatonin (MEL) may affect both the response to stressful events and the immune response. The pineal MEL and plasma MEL, corticosterone (B) and progesterone (P) levels were altered by several modes of stressors. In stressed animals the correlation between MEL and P secretion was observed during the light and dark period. MEL administered before transport stress was able to decrease the elevation of blood cortisol content in buffalo heifers. The superfusion of rat adrenal with MEL did not affect the B secretion but decreased its P content. Those results showed that in stressed animals MEL may alter adrenal steroid secretion. The study performed on human peripheral blood lymphocytes and tonsillar lymphocytes activated with concanavalin A (Con-A) showed that the stimulated proliferation was associated with the increased specific 2-(^{125}I)-MEL binding. The addition of MEL or 5-metoxytryptophol (5-MTO) decreased but 5-metoxytryptamine (5-MTA) increased the Con-A stimulated DNA synthesis in a dose-depending manner. The functional MEL receptor (Kd: 0.14 ± 0.03 nM; Bmax: 6.8 ± 1.5 fmol/mg P) was found on Con-A activated lymphocytes which binds 5-MTO but not by 5-MTA. These data indicate a direct participation of MEL in lymphoproliferation and lymphocyte function. In conclusion, MEL is capable to modulate the immune system as well as its endocrine response to stress.

PRE-TREATMENT PROGESTERONE DOSE ON THE TIMING OF THE LH SURGE IN EWES

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This experiment examined the effect of pre-treatment with two doses of intravaginal progesterone on the timing of the LH surge in intact and ovariectomised ewes. Ten intact ewes were given prostaglandin at the time of progesterone (1 CIDR-G) withdrawal to synchronise a follicular phase. Ten ovariectomised ewes were treated with steroids to mimic a normal follicular phase i.e. 10 days intravaginal progesterone (1 CIDR-G), withdrawn at time zero, followed by subcutaneous insertion of 2 Silastic implants (30 mm x 3.3 mm diameter) containing oestradiol at 22 h and a further 2 at 32 h. Blood samples were taken every two hours from 32 to 64 h. This regime was repeated for both groups but two CIDR-G were used. In the intact ewes, the timing of the LH surge was delayed, and more variable, after 1 CIDR-G was used compared to 2 CIDR-G, (48.4 ± 5.7 h vs. 40.5 ± 2.6 h, $P < 0.005$). In the ovariectomised ewes, there was no difference in the timing of the LH surge when 1 or 2 CIDR-G were used (37.8 ± 2.7 h vs. 39.5 ± 1.6 h, $P < 0.18$). The timing of the LH surge after 2 CIDR-G in intact ewes was similar to that after 1 CIDR-G in the ovariectomised ewes. In conclusion, the variable effect of progesterone in intact ewes probably influenced LH pulse frequency, the production of endogenous oestradiol and hence the timing of the LH surge.

OPIOID INVOLVEMENT IN THE CONTROL OF THE PIG PINEAL GLAND ACTIVITY - A PRELIMINARY STUDY

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The hypothesis that activity of the mammalian pineal gland may be modulated by opioid peptides is suggested by the results of some immunohistochemical and biochemical studies. The aim of the present study was to check the existence of opioidergic regulation in the pig pineal gland. An immunohistochemical method for demonstration of leu-enkephalin presence and an *in vitro* incubation technique for investigations of opioid agonists effects on melatonin secretion were employed. Two separate *in vitro* experiments were performed. In the exp. I slices of the pig pineal glands were incubated for 4 h in medium 199 containing morphine, morphine+naloxone, isoproterenol, isoproterenol+morphine, isoproterenol+morphine+naloxone. Morphine and naloxone were used at the concentration 10^{-4}M, isoproterenol at 10^{-5}M. In the experiment II morphiceptin (10^{-4}M) was used instead of morphine and aprotinin (60 μg/ml) was added to the medium. Numerous leu-enkephalin positive nerve fibers were found in the ependymal layer of the pineal recessus and in the subependymal layer. In other parts of the gland single, usually located in connective septa, immunoreactive fibers were found. In the *in vitro* studies, morphine increased basal and decreased isoproterenol-stimulated melatonin accumulation in the medium. Morphiceptin has only a slight inhibitory effect on isoproterenol-stimulated melatonin accumulation. The effects of morphine and morphiceptin were blocked by naloxone.

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MELATONIN REGULATION OF GONADOTROPHIN AND PROLACTIN SECRETION IN THE RAM: THE DUAL SITE HYPOTHESIS

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The "dual site" hypothesis proposes that melatonin acts at separate sites in the brain and pituitary gland to regulate gonadotrophin and prolactin secretion, and thus mediate effects of photoperiod on the reproductive axis. The pituitary gland is the proposed site of action of melatonin for prolactin regulation based on the observation that hypothalamo-pituitary disconnected rams continue to show normal patterns of prolactin secretion in response to changes in photoperiod and treatment with melatonin, in the absence of any demonstrable control by the hypothalamus. Gonadotrophin secretion was permanently low in these animals with no cyclical variation. The mediobasal hypothalamus (MBH) is the putative site for gonadotrophin regulation based the observation in both rams and ewes that micro-implants of melatonin placed in the MBH, but not in other sites in the brain and pituitary gland, were maximally effective at reversing the inhibitory effect of long days on FSH and LH secretion. Implants in the pars tuberalis (PT) were less effective or ineffective, precluding this tissue from having a key role in the neuroendocrine relay. Complementary studies in the Syrian hamster conducted by others, have shown that lesions of the MBH, particularly the dorsomedial hypothalamus, block the actions of melatonin on gonadotrophin secretion but not on prolactin secretion. The dual site hypothesis helps to explain how different mammals can have a similar timing for the seasonal prolactin cycle (all long day species), but a very different timing for the gonadotrophin cycle (long vs. short day species).

SEX-SPECIFIC EFFECTS OF MATERNAL STRESS ON STRESS-RESPONSIVENESS IN ADULT RAT OFFSPRING

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The offspring of dams exposed to stress during the last week of pregnancy show altered hypothalamo-pituitary-adrenocortical responses to stress. The effects may differ depending upon the gender of the offspring. We measured blood plasma corticosterone level and hypothalamic catecholamine content prior and 60 min following acute stress immobilization in 3-month-old male and female rat offspring whose mothers had been exposed to daily one-hour restraint immobilization stress during the 15-21st days of pregnancy. Maternal stress resulted in decrease of corticosterone response to acute stress in adult males, but not in females.Whereas basal corticosterone levels in intact and prenatally stressed males were similar, following acute immobilization corticosterone increased by 162% and 54%, respectively. In contrast, basal plasma corticosterone level in prenatally stressed females was higher compared with intact rats, however magnitude of stress-induced increases in corticosterone secretion in intact and prenatally stressed females was similar. Acute stress caused the lowering of hypothalamic noradrenaline concentration in intactrats of both genders as well as in prenatally stressed females. Such a response was not observed in prenatally stressed males. As a result of prenatal stress an increase of hypothalamic dopamine content was found in adult males. No gender differences in response of hypothalamic dopamine in all experimental groups were found.These results indicate that the maternal stress affects brain development in the offspring of both genders. The observed changes in stress-reactivity may be considered as manifestations of disorders in hormone-neurotransmitter imprinting of the neuroendocrine system.

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PHOTOPERIODIC CHANGES IN TESTICULAR ACTIVITY OF THE RAT-LIKE HAMSTER *(Cricetulus triton)*

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We have raised a closed colony of rat-like hamsters (*Cricetulus triton*) which were caught in Northern part of China since 1991. The body weight is about 170 g and body lenght from head to tail is about 20 cm. The details of the reproductive features such as the onset of puberty, sexual behavior and hormonal profiles during the estrus cycle, are still unknown. It has been considered that the rat-like hamster is a seasonal breeder. The present study was designed to determine the photoperiodicity of the reproductive functions and the role of the pineal gland in mediating the photoperiodic response in rat-like hamster. Males were exposed to an alternative condition of 8-week long days (14 h light/day) and short days (8 h light day). Some males were pinealectomized under long days before the animals were transferred to short days. Testis size was determined twice a week by measuring both long and short diameters. Intact male animals showed a testicular regression under short days and a recrudescence after transferring from short days to long days. Pinealectomized male animals showed no changes in testis size. These results indicate that the rat-like hamster is a long-day breeder, whose photoperiodicity is regulated by the pineal gland. The hamster could be a useful model for investigating the neuroendocrine mechanism for the seasonal breeding.

EFFECT OF TRANSPORT TIMING ON PITUITARY RESPONSIVENESS TO EXOGENOUS GnRH AND ON OESTRADIOL-INDUCED LH SURGES IN THE EWE

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This study examined the effect of transport timing on LH secretion. Oestrus was synchronized with progestin sponges and prostaglandin (PG). Twenty four hours after PG, 27 ewes received 50 μg oestradiol (E2) and 8 h later 3 injections of GnRH (500 ng) were given at 2 h intervals. The regimen was repeated with ewes transported for 8.5 h beginning either 7.5 h (Group 1; n = 18) or 5.5 h (Group 2; n = 9) after E2. The LH responses to the 2nd and 3rd GnRH injections were greater than the first (P<0.01). In Group 1, transport reduced the LH response to the 2nd GnRH (P<0.05) but not to first (P = 0.08) and third GnRH (P = 0.16). In Group 2, transport did not affect the LH response to any of the GnRH injections. The amplitude and duration of the LH surge remained the same but the onset time was significantly delayed (P<0.05) by transport in Group1 (24.0 ± 1.7 h vs. 26.3 ± 1.8 h) but not in Group 2 (27.8 ± 2.5 h vs. 26.4 ± 2.4 h). Transport significantly increased plasma cortisol (P<0.01) and progesterone (P<0.05) concentrations. In conclusion, the timing of the stressor is critical when considering the ability of transport to reduce the self-priming effect of GnRH and delay the LH surge.

MOLECULAR MECHANISMS INVOLVED IN THE ALTERATION OF NEURONS CONTROLLING THE REPRODUCTIVE FUNCTIONS

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The molecular events leading to LHRH biosynthesis and activity throughout the ovulatory cycle can be profoundly disturbed during prolonged and severe stressful conditions. Among the stress-related factors known for their inhibitory effects on the activity of LHRH neurons, interleukin-1 (IL-1) is probably the most potent one. Indeed, the hypothalamic release of LHRH during the afternoon of proestrus, as well as the plasma LH levels in gonadectomized male and female rats, are practically undetectable 2 h after the intracerebroventricular (i.c.v.) infusion of small doses of IL-1β. The LHRH biosynthesis in neurons located in the medial preoptic area (MPOA) is also decreased in animals i.c.v.infused with Il-1β an effect accompanied with a profound disruption of the ovulatory cycle. Our data also strongly support the hypothesis that IL-1β interferes with the LHRH neuronal activity by acting at the level of LHRH perikarya rather that at the level of LHRH nerve terminals located in the median eminence (ME). In fact, central treatment with IL-1β is able to completely inhibit the spontaneous expression of c-*fos* occurring in LHRH perikarya during the critical period of proestrus afternoon. Moreover, microinfusion of IL-1β bilaterally into the MPOA reduces LHRH release and interferes with the signal transduction within LHRH neurons in proestrus rats. These results indicate that the MPOA represents a target region for central IL-1β to modulate the activity of LHRH neurons projecting to the ME. It is important to point out that a putative relationship between the alteration of c-*fos* within LHRH neuron nuclei and the inhibition of hypothalamic LHRH release, has not been yet demonstrated. It is possible that IL-1β inhibits the endogenous expression of c-*fos*, which in turn may alter the LHRH gene transcription by a distinct and independent mechanisms controlling the LHRH release. Interestingly, central administration of CRF antagonists does not modify the IL-1β-induced inhibition of LHRH neuronal activity. It is permit to believe that CRF and IL-1 play a role, independent of each other, to alter the reproductive system at the level of LHRH cell bodies during specific challanges such as activation of the immune system in the case of IL-1. The fact that LHRH neurons do not seem to express neither CRF nor IL-1 receptors indicate that these peptides act *via* intermediate signals. Indeed, the recent cloning of various CRF receptor subtypes have provided new tools to investigate the CRFergic system in the brain and double-labeling techniques did not reveal any convincing positive signal for the mRNAs encoding either CRF or IL-1 receptors on LHRH cell bodies. However, still remains the possibility that both peptides act on cells located in close association with LHRH perikarya.

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THE PROTRACTED FORM OF LECIRELIN AS A TOOL FOR INFLUENCING THE OVARIAN ACTIVITY OF DOMESTIC ANIMALS

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Our work presents pharmacokinetic studies and the influence of administration of a protracted form of lecirelin (work-name Polygestran inj.) in treatment of post partal anoestria of cows after the 60th day post partum (29 cows), of the treatment of ovarian cysts (40 cows) and the induction of heat in small ruminants in the period of the seasonal anoestria (72 sheep and 26 goats). The new product was compared with non-protracted form of lecirelin (Supergestran inj. Ferring - Léčiva, Prague, Czech Republic) (post partal anoestria - 54 cows, ovarian cysts - 33 cows). Control group of small ruminants (this group included 29 sheep and 7 goats) was not treated at all. As for the treatment of the puerperally anoestria and ovarian cysts, the protracted form of lecirelin was more active than the traditional product Supergestran inj. In case of application of protracted form of lecirelin the time from the application to the first insemination was significantly shorter (anoestria - 17 days vs. 34.6 days, ovarian cysts - 28.3 days vs. 46.1 days). We have identified new indication area in biotechnological use of the protracted form of lecirelin - induction of the heat in small ruminants in the anoestrus season - the physiological oestrus started in 58% of sheep and in 69% of goats treated by Polygestran inj. In untreated animals the heat was detected in 14% of sheep and in 43% of goats.

THE EFFECT OF CATECHOLAMINE ON PRL SECRETION FROM THE PORCINE PITUITARY IN VITRO IN DEPENDENCE ON ESTROGEN STIMULATION *IN VIVO*

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The objective of this study was to determine what type of catecholamine receptors (α or β) is involved in the estrogen-dependent modulation of PRL secretion in the pig. Pituitary glands were removed from ovariectomized gilts (OVX) assigned to the following treatment groups: BE 0 (without estradiol benzoate pretreatment) and BE I or BEII (injection of BE at 30-36 h or 60-66 h before slaughter, respectively). Pituitary cells were diluted to 100,000 cells /ml with McCoys 5A medium and cultured at 37° C in 95%

air and 5% CO_2. Cultured medium was changed 48 h after seeding and subsequently pituitary cells were cultured (3.5 h) with: 10 nM, 100 nM i 1 μM phenylephrine (α-adrenergic agonist), 1 μM regitin (α-adrenergic blocker), 10 nM, 100 nM and 1 μM isoproterenol (β-adrenergic agonist), 1 μM propranolol (β-adrenergic blocker). In BE 0 group phenylephrine (10 nM and 1 μM) significantly stimulated prolactin release, while both isoproterenol and propranolol significantly inhibited prolactin secretion. In the group BE I addition of phenylephrine together with regitin or isoproterenol (1 μM) caused increase in PRL secretion. In the group BE II PRL release was stimulated by both phenylephrine (100 nM) and regitin. Also isoproterenol (10 nM) and isoproterenol added together with propanolol enhanced PRL secretion. The obtained results indicate that stimulation of the α-adrenergic receptors may enhance PRL secretion from pituitary cells of BE 0 group and BE II. Additionally, a blockade of α-adrenergic receptors also increased PRL secretion in group BE II. β-adrenergic agonists stimulated PRL secretion in BE I and BE II groups.

CENTRAL ALPHA-ADRENERGIC STIMULATION OF CORTISOL SECRETION IN SHEEP

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The paraventricular nucleus of the hypothalamus contains alpha-1-adrenergic receptors and is well innervated by (nor)adrenergic fibres. The present experiment examined the role of these receptors in the control of cortisol secretion. Guide cannulae (18 gauge) were placed into the dorsal part of the third ventricle of four mature Suffolk x Welsh ewes at least 2 months prior to the experiment and the animals were acclimatized to human contact. The selective alpha-1-adrenergic receptor agonist methoxamine (1 mg in 50 ml saline) or saline alone, was loaded into a 20 gauge cannula and Silastic tubing. This assembly was then placed down the guide cannula one hour before the start of blood sampling. Blood (2 ml) from the jugular vein was taken every 10 min for 2 h before methoxamine or saline was administered into the third ventricle (over 30 s) and for 4 h afterwards. Plasma cortisol was measured by radioimmunoassay after ethanol extraction. Mean plasma cortisol concentrations were significantly increased for the hour after methoxamine injection when compared to saline administration alone or compared to the hour prior to administration ($P<0.05$). These data support the hypothesis that stimulation of hypothalamic alpha-1-adrenergic receptors causes cortisol release. This confirms our preliminary data to suggest that cortisol secretion in response to intracerebroventricular noradrenaline is mediated *via* alpha-1-adrenergic receptors.

POSTNATAL DEVELOPMENT OF MELATONIN BINDING SITES IN THE BRAIN AND PRIMARY LYMPHOID GLANDS IN CHICKEN

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The aim of present study was to demonstrate and characterize melatonin (MEL) binding sites, and their changes within central nervous system and primary lymphoid organs in chicken during early posthatching life. Saturation and kinetic assays were performed using 2-[125I]MEL in membrane preparations isolated from respective tissues, taken from 2- to 28-day old cockerels, kept from hatching under L:D=12:12 conditions, and sacrificed at mid-day period. Binding of 2-[125I]MEL to the membrane preparations fulfils all criteria for binding to one type of receptor site with a high affinity and low capacity. MEL binding sites density was much higher in the central nervous system than in lymphoid organs. Of the brain structures examined, the highest MEL binding was in the optic tecta (OPT), next in hypothalamus (HPL), and the lowest in telencephalon (TEL). MEL binding in the bursa of Fabricius (BF) was low, but still several fold higher than that in the thymus (T). Two developmental patterns of the MEL binding density could be distinguished: (1) changes in the binding sites number during first week of postnatal development, observed in TEL (decrease) and T (increase), following by relatively constant level, and (2) continuous developmental changes observed in OPT, HPL and BF from 2-4 to 21 day of life. The physiological relevance of these changes in MEL binding remains to be elucidated.

THE EXPRESSION OF THE MELATONIN RECEPTOR IN THE HUMAN BRAIN

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Melatonin (MEL) is the main hormonal product of the vertebrate pineal gland. Its synthesis is restricted to the scotophase of the light-dark cycle. The nocturnal MEL peak is interpreted by the responsive elements in the CNS as a coding signal for daylengt and MEL is now considered an important endogenous *Zeitgeber* involved in the circadian timing. MEL triggers its effects through high-affinity, G-protein coupled receptors. The adult human brain has been a subject of a limited number of studies regarding the distribution of the MEL receptor. MEL binding sites suggestive of a functional MEL receptor have been described in the hypothalamic suprachiasmatic nuclei and, in limited cases, in the pituitary pars tuberalis. The external molecular layer of the cerebellum appeared to express the highest density MEL receptors. Cloning and sequencing analysis of the full-length coding region isolated by RT-PCR from human cerebellar specimens revealed that the receptor protein is encoded by a transcript identical to that recently cloned from the hypothalamus (Mel_{1a}). *In situ* hybridization with an antisense cRNA-probe demonstrated that the receptor mRNA is localized in the cerebellar granule cells. Partial mapping of the messenger by RT-PCR with Mel_{1a} specific primers in different areas of the human brain disclosed a quite widespread distribution of the transcript, although expressed at very low levels. Semi-quantitative comparisons of different brain regions resulted in the following relative mRNA abundance: cerebellum > occipital cortex/parietal cortex temporal cortex thalamus frontal cortex/hippocampus.

MATERNAL STRESS-INDUCED CHANGES IN TESTOSTERONE METABOLISM IN DISCRETE BRAIN STRUCTURES OF RAT OFFSPRING

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We have already reported that maternal immobilization stress during the last third of pregnancy resulted in disappearance of sexual differences in hypothalamic testosterone metabolism in 10 day-old rat off-spring. Taking into account the location of centers controlling male and female sexual behavior and gonadotropin secretion pattern we investigated the aromatase (AA) and 5 α-reductase (RA) activities in the preoptic area (POA) and mediobasal hypothalamus (MBH) of prenatally stressed 10- and 90 day-old male and female rats in the present study. The enzyme activities were measured by product isolation assay. In the POA of intact 10 day-old animals distinct sex differences of AA were observed. Prenatal stress removed the sexual dimorphism in the POA AA due to its 1.6-fold decrease in males. Neither sexual nor stress-induced differences in AA were found in the MBH. Unlike AA RA was higher in the POA of 10 day-old intact females. In prenatally stressed males POA RA increased 3 times and became higher than in females. In the MBH prenatal stress induced the appearance of male-female differences in RA which were absent in intact 10 day-old rats. In 90 day-old offspring sexual dimorphism of AA was not observed both in intact and prenatally stressed animals. In the POA of intact mature rats there was significant male-female difference in RA. As well as in 10 day-old prenatally stressed males in the mature ones the RA rise in the POA was found. The MBH RA was not affected by prenatal stress in mature rats of both genders. The data obtained suggest that the developmental changes both of brain steroid aromatase and 5 α-reductase activities may contribute to the maternal stress-induced alterations of sexual brain differentiation and gonadotropin secretion control.

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ESTROGEN MODULATES EFFECTS OF GLUTAMATE ON *IN VITRO* LHRH RELEASE BY ALTERING NITRIC OXIDE ACTION IN FEMALE RATS

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The arcuate nucleus (ARC) is considered to be one of the target sates of estrogen feedback. Nevertheless, it is not well understood the feedback mechanism in the ARC. Our recent study demonstrated that estrogen modulates glutamate-induced *in vitro* LHRH release from ARC-median eminence (ME) fragments in female rats. Nitric oxide (NO) is one of the factors regulating LHRH release. In the present study, the effect of NO synthase inhibitor (NSI) on glutamate-induced *in vitro* LHRH release from ARC-ME or ME fragments taken from ovariectomized (OVX) or OVX and estradiol-primed (OVX+E_2) rats was examined to determine whether estrogen modulates the effect of NO on glutamate-induced *in vitro* LHRH release. In the ME fragment, NSI enhanced glutamate-induced *in vitro* LHRH release taken from both OVX and OVX+E_2 rats. Although NO has an inhibitory effect in ARC-ME fragments taken from OVX rats as well as ME fragments, glutamate-induced LHRH release from ARC-ME fragments was completely suppressed by NSI in OVX+E_2 rats. These results indicate that estrogen modulates glutamate-induced LHRH release from the ARC-ME fragment by altering NO action mediating the effect of glutamate.

THE BRAIN MECHANISM MEDIATING FASTING-INDUCED SUPPRESSION OF PULSATILE LH RELEASE IN THE MALE RAT

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The present study was aimed to determine (1) whether fasting-induced suppression of pulsatile LH secretion in male rats is testosterone-dependent, (2) whether the PVN is involved in the mechanism responsible for fasting-induced inhibition of LH release, and (3) where the feedback site(s) of gonadal steroids during fasting are. Pulsatile LH secretion was suppressed in a testosterone-dependent manner after 48-h of fasting. Expression of c-fos was induced in the PVN only in testosterone-treated castrated males but not in castrated not treated animals. Furthermore, PVN lesion prevented the suppressing effect of fasting on pulsatile LH release. These results suggest that the PVN is involved in the fasting-induced suppression of LH secretion in the testosterone -implanted castrated rats. In addition, local implantation of testosterone or estradiol into the PVN did not reverse the LH suppression during fasting in castrated male rats, suggesting that other region(s) in the brain than the PVN could be the gonadal steroid feedback site(s) responsible for the fasting-induced inhibition of pulsatile LH secretion in male rats.

CHANGES IN PROLACTINE SECRETION RHYTHM, TESTIS SIZE AND BODY WEIGHT OF POLISH LONGWOOL RAMS IN FIRST THREE YEARS OF LIFE

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The increase of prolactine concentration in blood found in many sheep breeds in spring-summer season, followed by its drop in autumn-winter season, points to the contribution of that hormone in controlling the reproduction season. However, the studies, performed by Howles et al. (1) and Lincoln (2), has not confirmed the above connection of prolactine with seasonality in reproduction. Also, the role of that hormone in the growth and development process of ruminants has not been explained yet. Thus, the aim of present studies was to establish the correlations between the blood prolactine level and the testis size and the increase of body weight in rams in first three years of life. The studies were performed with 10 rams born in January. The animals were kept in natural conditions, and the ratio of windows to floor space was 1:17. Beginning with the fifth month of life (May) up to the end of the third year of life, the body weight of rams and the measurements of testis circumference, as well the blood samples were taken three times a month. The results obtained are given in form of the average monthly values. It was found, that the average blood concentration of prolactine has been increasing in the first year with the age and the length of day, with the highest value in August (119.0 ng/ml). Starting with September, a clear decrease in the level of that hormone has been occurring, reaching the lowest values in November and December, 16.5 and 22.0 ng/ml, respectively. In the second and third yaer of life, the highest level of prolactine was found in spring and summer months, and the lowest one in autumn and winter months, alike.The circumference of testicles has been shaped in a different manner in these months, increasing regulary from 16 cm in the fifth month to 30 cm in the eleventh month of life. A drop of testis circumference has been occurring from December to May, and an increase from June to November. The body weight has been increasing from 23.5 kg in the fifth month to 95 kg in the thirty-sixth month of life. The coefficients of correlation between the level of prolactine and the circumference of testicles were negative in all years, and amounted to -0.360; -0.130 and -0.343 in the first, second and third year of life, respectively. Correlation between the level of prolactine and the body weight has been ranging from significantly negative in the first year of life (-0.424) to significantly positive in the third year of life (0.123). The results obtained indicate to: (1) the occurence of different circannual rhythms in the secretion of prolactine and in the size od testis, and to the regular increase of body weight with age of rams; (2) the irrelevance between the seasonal variations in the prolactine concentration and the changes in testis size and the increases of body weight in the time of growth and development of rams.

1. Howels et al. (1982) J. Reprod. Fertil. 65: 439-444.
2. Lincoln G.A. (1990) J. Reprod. Fertil. 90: 285-295.