Increasing evidence, mainly from rodents, suggests that the predominant estrogen receptor (ER) in arteries is the newly-described ERbeta. We have investigated the expression of the two ERs in baboon carotid artery before and after denudation injury. Prior to denudation, both full length receptors were detected in semiquantitative RT-PCR; in addition two ERalpha but no ERbeta splicing variants were found. After denudation, ERbeta mRNA increased five-fold and declined, whereas ERalpha mRNA expression remained low. Prior to and after denudation, two ERalpha-specific antibodies showed no reaction with the vessel wall. Instead, two affinity purified antisera to ERbeta demonstrated a weak but distinct reaction over vascular smooth muscle cells with predenudation specimens, escalating post-denudation and declining thereafter. The results suggest that selective targeting to ERbeta should be attempted when designing estrogen-based vasculoprotective drug therapies devoid of uterotrophic side effects.


The therapeutic efficacy and antiovulatory properties of non-steroidal anti-inflammatory drugs (NSAIDs) is attributed to their ability to suppress prostaglandin endoperoxide synthase (PGS) activity. Given the likely role of interleukin (IL)-1 in the inflammatory (and probably the ovulatory) process, we set out to evaluate whether the antiovulatory property of NSAIDs is attributable, in part, to the inhibition of ovarian IL-1 action. Whole ovarian dispersates from immature rats were cultured under serum-free conditions in the absence or presence of the indicated agents. At the conclusion of the culture period, total RNA was extracted and probed for transcripts corresponding to PGS-1, PGS-2, IL-1[beta], IL-1 receptor antagonist (IL-1RA) or type I IL-1 receptor (IL-1R) by a solution hybridization/ribonuclease protection assay. Treatment with indomethacin was without significant effect on the early (1 h) response to IL-1[beta]; however, it led to complete and highly significant dose-dependent blockade of the late (48 h) response to IL-1[beta] as assessed in terms of PGS-2 transcripts, proteins and activity. The addition of PGE2 to cells augmented the ability of IL-1[beta] to upregulate PGS-2 transcripts. Moreover, the addition of PGE2 to indomethacin-treated cells all but reversed the ability of indomethacin to suppress the IL-1[beta] effect at both the PGS-2 transcript and protein levels. The upregulation by IL-1 of IL-
1[\beta], IL-1R and IL-1RA transcripts was similarly inhibited by indomethacin. Taken together, these observations suggest that the anti-ovulatory property of NSAIDs may be due, in part, to blockade of the late, prostanoid-dependent component of ovarian IL-1 action.


http://www.sciencedirect.com/science/article/B6T3G-47GGRXR-F/2/7811f0f6159449df2f1a8cfbd1a964734

Hypothalamic dopamine (DA), acting at DA D2-receptors (D2-R) on pituitary target cells, mediates peptide release and biosynthesis of rat pituitary anterior lobe (AL) prolactin, and neurointermediate lobe (NIL) pro-opiomelanocortin (POMC). We were interested in determining if dopamine agonists and antagonists were capable of modifying D2-R gene expression in these pituitary cells. Utilizing the recently published sequence of the rat D2-R, we isolated a rat D2-R cDNA clone by polymerase chain reaction, and have synthesized RNA probes to quantitate levels of D2-R mRNA by solution hybridization/nuclease protection assay. We report here that 5-day administration of the DA antagonist haloperidol led to significant increase in both D2-R mRNA and POMC mRNA in the NIL; the DA agonist bromocriptine caused a significant decrease in NIL POMC mRNA with no parallel change in D2-R mRNA. In contrast, no significant changes in D2-R mRNA in AL were observed following treatment with either the DA agonist or antagonist. These data provide evidence for tissue-specific regulation of D2-R mRNA in response to dopaminergic manipulation.


http://www.sciencedirect.com/science/article/B6T3G-41JM913-D/2/623b23032e74b10b54a473808932ba74

Complementary DNAs for the open reading frames of the chicken, Xenopus and zebrafish StAR homologs were cloned along with a partial cDNA of the zebrafish homolog to MLN64, a StAR-related protein. A comparison of the amino acid sequences of piscine, amphibian, avian and mammalian STARS, indicates strong conservation of the protein across divergent vertebrate groups. On Northern blots probed with species specific StAR cDNAs, expression of StAR transcripts was observed in the ovary and adrenal of chicken, and the ovary, testis, kidney and head of zebrafish. The expression of StAR mRNA in various compartments of the hen ovary was consistent with the results of past studies on steroidogenesis; expression was first observed in follicles selected into the preovulatory hierarchy and was greatest in the largest preovulatory follicle. The expression of StAR mRNA was also consistent with aromatase expression in zebrafish ovaries. The conserved deduced protein sequence and expression pattern of StAR transcripts in chicken and zebrafish tissues, strongly suggest that StAR is also involved in the regulation of steroidogenesis in nonmammalian vertebrates.

The UDP-glucuronosyltransferase (EC 2.4.1.17) enzymes transform many lipophilic compounds to more water-soluble products via conjugation with glucuronic acid. This conversion is responsible for enhancing the excretion of endogenous aglycones such as steroids. To date, several distinct isoforms of steroid UDP-glucuronosyltransferases (UGTs) have been isolated in the human liver. Among these UGTs, UGT2B7 is specific for estriol and 3,4-catechol estrogens, UGT2B15 glucuronidates 17[beta]-hydroxy-C19 steroids while UGT2B10 has as yet an undescribed activity. To further demonstrate the presence of UGTs in peripheral tissues we studied the expression of these enzymes in human prostate hyperplastic tissue and the LNCaP cell line. Metabolism studies using intact LNCaP cells in culture indicate the presence of UGT activities involved in the glucuronidation of 3[alpha]-hydroxysteroids (androsterone) and 17[beta]-hydroxysteroids (testosterone and dihydrotestosterone). Northern blot analysis of poly(A+) RNA from LNCaP cells and prostate using a UGT2B15 cDNA probe revealed two bands of 2.0 and 2.3 kb. In order to identify more specifically the mRNAs detected in Northern blot analysis we used RNase protection and RT-PCR assays. The relatively high expression of UGT2B10 and UGT2B15 in LNCaP cells was confirmed by RNase protection and RT-PCR, although, these approaches did not allow detection of UGT2B7 transcripts. Our studies demonstrate the presence of two UGT activities and at least two types of UGT transcripts in both the human prostate and the LNCaP cells.


http://www.sciencedirect.com/science/article/B6T3G-46THXCK-5/2/3ba70e8424422115541d89cf6e2f0ccc

Vitellogenin (Vtg) and estrogen receptor (ER) gene expression levels were measured in largemouth bass to evaluate the activation of the ER-mediated pathway by estradiol (E2). Single injections of E2 ranging from 0.0005 to 5 mg/kg up-regulated plasma Vtg in a dose-dependent manner. Vtg and ER mRNAs were measured using partial cDNA sequences corresponding to the C-terminal domain for Vtg and the ligand-binding domain of ER[alpha] sequences. After acute E2-exposures (2 mg/kg), Vtg and ER mRNAs and plasma Vtg levels peaked after 2 days. The rate of ER mRNA accumulation peaked 36-42 h earlier than Vtg mRNA. The expression window for ER defines the primary response to E2 in largemouth bass and that for Vtg a delayed primary response. The specific effect of E2 on other estrogen-regulated genes was tested during these same time windows using differential display RT-PCR. Specific up-regulated genes that are expressed in the same time window as Vtg were ERp72 (a membrane-bound disulfide isomerase) and a gene with homology to an expressed gene identified in zebrafish. Genes that were expressed in a pattern that mimics the ER include the gene for zona radiata protein ZP2, and a gene with homology to an expressed gene found in winter flounder. One gene for fibrinogen [gamma] was down-regulated and an unidentified gene was transiently up-regulated after 12 h of exposure and returned to basal levels by 48 h. Taken together these studies indicate that the acute molecular response to E2 involves a complex network of responses over time.


http://www.sciencedirect.com/science/article/B6T3G-3R7B1MD-2/2/f59e4986d3415a349ffa4ae587cdf40b
We have previously shown that thyroid stimulating hormone-[beta] (TSH[beta]) mRNA levels are modulated by vitamin A status in vivo and using transient transfection, that suppression of rat TSH[beta] gene promoter activity by all-trans retinoic acid (RA) requires RA receptor (RAR) and retinoid X receptor (RXR). In this paper we have used deletion analysis to delineate the sequences of the rTSH[beta] gene involved in RA regulation, their relationship to the rTSH[beta] gene negative thyroid hormone response elements and the retinoid receptor species that interact with these sequences. Using transient transfection in CV-1 cells, we found that the -204/+9 region of the rat TSH[beta] gene, when fused to a luciferase reporter, was sufficient for suppression by all-trans-RA in the presence of RAR/RXR. Thus, regulation by RA did not involve the major rTSH[beta] negative TRE located between +15 and +43. Mutational analysis also showed that the minor rTSH[beta] negative TRE between -11 and +5 was not required by suppression by RA. However, in a heterologous promoter this sequence element acted as a strong positive RARE. The combination of RA and T3 treatment caused synergistic inhibition of rat TSH[beta] gene expression in the presence of RAR/RXR and TR. EMSA analysis demonstrated that the -204/-79 sequence binds RAR/RXR heterodimer. Therefore, we conclude that there are separate response elements for RA and T3 on the rat TSH[beta] gene, that the RARE binds RAR/RXR heterodimer and that RA and T3 interact functionally via these elements in the negative regulation of rat TSH[beta] gene expression.


http://www.sciencedirect.com/science/article/B6T3G-430NR88-F/2/3dc4d72b66fc7cb61108daccbd42f59e

mRNA differential display-PCR analysis was used to perform a systematic screening of Somatostatin (SS)-regulated genes in the human prostatic carcinoma cell line LNCaP (Lymph Node Carcinoma of the Prostate). A 170 bp fragment was shown to be up-regulated by SS. Sequence analysis of this fragment revealed its homology with the human Topoisomerase II Alpha gene. Up-regulation of Topoisomerase II Alpha was confirmed by Northern blot hybridisation and was induced by the same dose of SS (1 nM) earlier demonstrated to inhibit LNCaP cell growth. Furthermore, SS possible effects on timing, as well as concentration of Topoisomerase II Alpha along the different phases of the cell cycle were investigated. To this purpose changes in the enzyme protein concentration in response to SS were assessed in synchronised LNCaP cells. The hormone was shown to exert a perturbing effect on both parameters considered, possibly related to its inhibitory action on LNCaP cell replication.


http://www.sciencedirect.com/science/article/B6T3G-481N1T0-3/2/b7bbafdf099c208fc10751e81385a14a7

Hyperfunctioning thyroid nodules are characterized by the presence of spontaneous somatic mutations responsible for constitutive activation of the cAMP pathway. However, alterations affecting other elements of the cAMP signaling system may counteract the effects of the mutations. In this study, the expression of the adenylyl cyclase (AC) types III and VI was investigated by Western blot in 18 hyperfunctioning thyroid nodules; in 12 samples, we also assessed the presence of TSH receptor (TSHR) or gsp mutations and levels of AC VI and III mRNA. We found that the expression of nodular AC VI (but not AC III) was significantly lower (85.1% of normal, P=0.014) than the expression of both adenylyl cyclase types of perinodular tissue from the same patients. Slightly, but not significant differences were detected in nodules
with or without mutations and AC protein levels generally showed correlation with the levels of the transcripts detected by RT-PCR. In addition, AC III and AC VI expression levels within a given nodule were characterized by a significant positive correlation. These findings indicate that a diminished expression of AC type VI may be part of the mechanisms occurring in the hyperfunctioning nodules, independently of the presence of TSHR or gsp mutations, which influence the resulting phenotype.


http://www.sciencedirect.com/science/article/B6T3G-3TGVJSC-8/2/5fc2875c10aea84df387c9083beff6

Type II 5'-Deiodinase (5'DII) is a key element in the maintenance of peripheral thyroid hormone homeostasis through the regulation of local T4 to T3 conversion in pituitary, brain, brown adipose tissue and placenta. The cDNA containing the coding region of the human 5'DII (HDII) has been recently cloned from infant brain. In the present paper we report the genomic structure, chromosomal localization and restriction map of the coding region of HDII. The presence of a single intron located at codon 75 was demonstrated using a PCR-based strategy; the exon-intron junctions were then cloned and partially sequenced. Chromosomal localization was performed by radiation hybrid mapping. This study demonstrated that the entire coding region of the HDII gene is contained in two exons spliced at codon 75 by a 7.4 Kb intron and that the HDII chromosomal location is 14q24.3. These data will allow further studies of the role of HDII in the pathophysiology of thyroid homeostasis.


http://www.sciencedirect.com/science/article/B6T3G-42SGHHC-1/2/88d70912765a8b7c23d0ea865782b78

The complementary DNA (cDNA) encoding pituitary thyroid stimulating hormone beta subunit (TSH-[beta]) of bighead carp was cloned and regulation of its gene expression was investigated for understanding phylogenetic divergence and evolution of TSH molecule. The cDNA was obtained from bighead carp pituitary total RNA by reverse transcription and polymerase chain reaction. Oligonucleotide primers were designed from the sequence of common carp. The full length sequence was then obtained by 3' and 5' rapid amplification of cDNA ends (RACE). The full-length sequence consisting of 3' and 5' untranslated regions was 585 bp long. The predicted amino acid sequence consisted of a signal peptide of 19 amino acid residues and a mature TSH [beta] subunit protein of 131 residues. The coding sequences of the cDNAs showed variable percentage homologies with those of other teleosts and vertebrate species. The predicted amino acid sequence shared 71% identity with rainbow trout and salmon, 90% with goldfish, 50% with eel and 94% with common carp in the mature protein region. The percentages of identity in the same region in comparison with bovine, porcine, rat, mouse, human and chicken were only 39, 42, 41, 40, 45 and 46%, respectively. TSH [beta] mRNA expression was found only in the pituitary tissue out of other tissues tested as testis, muscle, brain and heart. For the first time, thyrotropin releasing hormone (TRH) and thyroxine (T4) effects on pituitary TSH mRNA expression were tested in teleosts under in vitro conditions. TRH treatment on pituitary cells increased TSH [beta] mRNA level, while T4 treatment decreased TSH [beta] mRNA level. The present study provides a direct evidence, for the first time that TRH directly upregulates TSH [beta] gene expression in teleosts.

http://www.sciencedirect.com/science/article/B6T3G-3XM2M1R-C/2/be232578da87b1a8294f1275adeb9fb4

To examine activity of estrogen receptor-beta (ER[beta]) independently of estrogen receptor-alpha (ER[alpha]), retrovirus-mediated gene transfer was used to insert rat ER[beta] into a rat fibroblast cell line (rat-1) that does not ordinarily express ER. Stable expression of ER[beta] in rat-1 cells was validated and then characterized by reverse-transcription polymerase chain-reaction (RT-PCR) analysis to examine the effects of estradiol (E2) treatment on expression of specific target mRNAs. Results were compared with rat-1 cells and a previously constructed rat-1+ER[alpha] cell line. Progesterone receptor mRNA was not detected in rat-1 cells and was induced by E2 in both rat-1+ER[alpha] and rat-1+ER[beta] cells. Treatment with E2 resulted in an increased rate of cell proliferation (P<0.05) in rat-1+ER[alpha] cells, but not in rat-1 or rat-1+ER[beta] cells. Data confirm studies using transient ER expression demonstrating that ER[alpha] and ER[beta] have both discrete and overlapping activity within the same cell type in the presence of the same ligand.


http://www.sciencedirect.com/science/article/B6T3G-47MCKGT-N0/2/5303840e55504f7a11c9cdae3daefaca

We have applied the polymerase chain reaction (PCR) and single-strand conformation polymorphism analysis (SSCP) to detect activating mutations in the Gs[alpha] subunit gene, amplifying genomic DNA extracted from growth hormone (GH)- and GH/prolactin (PRL)-secreting human pituitary tumors. Of 15 tumors tested six contained mutations in the analyzed regions of the Gs[alpha]. SSCP analysis revealed band shift in exon 8 in four GH- and in one GH/PRL-secreting tumors, and in exon 9 in one GH/PRL-secreting tumor. Direct sequencing of PCR reaction products identified the mutations as R201-H, R201-S and R201-C in exon 8 and Q227-L in exon 9. These results show the efficacy of PCR/SSCP analysis in the detection of G protein mutations and extend the generalization that these sites are hot spots in tumor-inducing mutations.


http://www.sciencedirect.com/science/article/B6T3G-441N4PC-3/2/bb2f89492c2a90db09b03dbac3e71fc

Triplet repeat base pair amplification is the basis for a number of prevalent genetic diseases such as Huntington's, Fragile X, Myotonic Dystrophy and others. We have chosen to investigate the use of PCR to amplify a portion of the Huntington's gene in single cells in order to develop a clinical test system for preimplantation genetic diagnosis (PGD). Amplification of CAG triplet repeat sequences poses difficulties due to resistance of GC melting for amplification. Special
PCR modifications are necessary to carry out the amplification of GC rich areas found in most triple base pair expansions. We have used a modified polymerase chain reaction (PCR) protocol to amplify the expanded repeat sequence of the Huntington's gene with satisfactory efficiency. Detection of the amplified expanded CAG repeats is shown to be possible using both agarose gel electrophoresis and high definition denaturing high pressure liquid (DHPLC) chromatography. The incidence of allele dropout (ADO) is documented.


In the presence of retinoic acid (RA), F9 murine teratocarcinoma cells differentiate into cells resembling the extra-embryonic endoderm of the early mouse embryo. Using differential hybridization, we have cloned and characterized six cDNAs corresponding to mRNAs that exhibit reduced expression in F9 cells following RA treatment. Two of these cDNAs encode novel genes (REX-2 and REX-3). The other isolated cDNAs encode genes that have been previously described in other contexts: 1-4 (cyclin D3); 2-10 (pyruvate kinase); 2-12 (glutathione S-transferase); and 2-17 (GLUT 3). The mRNA levels of these genes are reduced by RA or RA plus theophylline and cAMP (RACT) only after 48 h of treatment, and continue to decrease at 96 h. The half-lives of these mRNAs are not changed by RA treatment, indicating that these mRNAs may be regulated through a transcriptional mechanism. In isoleucine-deprived cells, which are growth arrested but do not differentiate, the steady state mRNA levels of genes Rex 2, Rex 3, pyruvate kinase and GLUT 3 are not reduced, in contrast to cyclin D3 and glutathione S-transferase. The expression of the REX-2, REX-3, pyruvate kinase, glutathione S-transferase and GLUT 3 genes is reduced by RACT to the same extent in F9 RAR[gamma] -/- and RAR[alpha]-/- lines as in F9-Wt. In contrast, cyclin D3 exhibits lower mRNA expression in F9 RAR[gamma] -/- and RAR[alpha]-/- stem cells, and this mRNA is not decreased by RACT treatment. Overexpression of cyclin D3 blocks the RA-induced growth arrest of F9 cells, indicating that the downregulation of this gene following RA treatment may constitute a necessary step in the cascade of events leading to growth inhibition by RA.


Single cell genetic analysis is generally performed using PCR and FISH. Until recently, FISH has been the method of choice. FISH however is expensive, has significant misdiagnosis rates, can result in interpretation difficulties and is labour intensive making it unsuitable for high throughput processing. Recently fluorescent PCR reliability has increased to levels at or surpassing FISH whilst maintaining low cost. However, PCR accuracy has been a concern due to allelic dropout. Multiplex PCR can now increase accuracy by using multiple markers for each chromosome to firstly provide diagnosis if markers fail and/or secondly confirm diagnosis. We compare a variety of diagnostic methods and demonstrate for the first time a multiplex PCR system providing simultaneous diagnosis and confirmation of the major aneuploidy chromosomes (21, 18, 13) and sex as well as DNA fingerprint in single cells. We also discuss the implications of using PCR for aneuploidy screening in preimplantation genetic diagnosis.

http://www.sciencedirect.com/science/article/B6T3G-417ND93-4/2/6305660ecd7eae390a1bd41917c7fb26

A partial cDNA encoding for the C-terminus of vitellogenin (VTG) was cloned from liver of Sparus aurata male treated with 17[beta]-estradiol (E2). E2 treatment of S. aurata males resulted in increased synthesis and secretion of VTG protein into the plasma, determined by a specific enzyme-linked immunosorbent assay (ELISA) in a time-dependent manner. While VTG mRNA was induced by E2 treatment, transthyretin (TTR) mRNA levels were reduced. These data provide the first demonstration that estrogen exhibits contrasting effect on VTG and on TTR gene expression in teleosts.


http://www.sciencedirect.com/science/article/B6T3G-42D2C93-9/2/b163d07c83f0dd8bb843999c3b1bf580

The action of follicle-stimulating hormone (FSH) in spermatogenesis is regulated at a fundamental level by controlling the number of competent receptors present at the surface of Sertoli cells. By controlling the number of receptors, the cell is able to modulate the timing and magnitude of subsequent signal transduction in response to FSH. One mechanism of control is the down-regulation of the steady state levels of the FSH receptor gene after exposure to FSH or agents that stimulate or prolong the cAMP signal transduction cascade (homologous down-regulation) in Sertoli cells. The goals of this study were to examine possible mechanisms involved in the down-regulation of mRNA levels of this gene. Analysis of transcription and processing by a PCR-based assay showed that treatment of Sertoli cells with FSH caused at least a 50% reduction of hnRNA for the FSH receptor gene. Reporter genes controlled by 5' flanking sequences of the FSH receptor gene that were transiently transfected into Sertoli cells were not down-regulated. In electrophoretic mobility shift assays (EMSA), cAMP-inducible nuclear protein complex containing c-Fos formed on the activator protein-1/cAMP responsive element-like site located at -216 to -210 in the promoter of the rat FSH receptor gene. We concluded from this study that there was no evidence for the putative role of ICER in the down-regulation of the FSH receptor promoter. In addition, the FSH-induced down-regulation of the transcription of the FSH receptor gene in Sertoli cells was prevented by the treatment of Sertoli cells with trichostatin A prior to the addition of FSH. This experiment coupled with other observations suggested that the down-regulation may be mediated by changes in chromatin structure.


http://www.sciencedirect.com/science/article/B6T3G-40XWF2-4/2/4b0ba37ce58b6c2d8bf1b514467f77b3
Cell-cell interactions are crucial for the proper functioning of endocrine glands. We recently demonstrated that interactions of chromaffin and cortical cells are important for adrenocortical steroidogenesis. However, the molecular mechanisms have not been elucidated and it is unclear if this involves acute and/or chronic processes. By Northern analysis and the quantitative technique of TaqMan PCR we investigated whether chromaffin cells influence the regulation of StAR and the peripheral benzodiazepine receptor (PBR), both required for the rate-limiting step, the delivery of cholesterol to the inner mitochondrial membrane. StAR mRNA levels in bovine adrenocortical cells were increased by incubation with chromaffin cell-conditioned medium (CCM). Short-term treatment for 4 h resulted in a greater stimulation (229±29% of basal, mean±SEM) than did longer incubation times of 8 h and 5 days (159±13 and 177±24%). Neither short nor a long-term treatment affected PBR expression. Consistently, the major secretion of chromaffin cells, epinephrine dose-dependently stimulated StAR expression with no effect on PBR mRNA. In conclusion, adrenomedullary secretory products are not necessary for the maintenance of PBR expression but facilitate steroid biosynthesis by increasing StAR mRNA expression and therefore can account for an ACTH-independent regulation of the rate-limiting step in steroidogenesis.


http://www.sciencedirect.com/science/article/B6T3G-3RSG33W-7/2/aa6cb501a8cc437099d41b002908aefe

In order to obtain homologous follicle-stimulating hormone (FSH) for in vivo and in vitro studies in the rat, rat recombinant (rec) FSH was produced in Chinese Hamster Ovary (CHO) cells. The synthesized rat recFSH was purified and subjected to physico-chemical and biological characterization, including a comparison with two rat pituitary (pit) and reference preparations (NIDDK-rFSH I-8 and NIDDK-rFSH-RP2) as well as with human recFSH (Org 32489). The molecular masses of rat recFSH and human recFSH were determined by SDS-polyacrylamide (SDS-PAGE) and were found to be similar, about 40 kD. The pl distribution of rat recFSH is similar to rat pitFSH, and slightly more acidic than human recFSH (3.6-5.6 vs 3.9-5.5, respectively) as determined by isoelectric focussing in immobilized pH gradients. Rat recFSH displayed dose-response curves parallel and in the same dose range as the rat pitFSH in receptor binding and in vitro bioassays. However, the in vivo activities of rat recFSH and rat pitFSH were 8824 and 3051 IU/mg, respectively, determined by the Steelman Poehley assay. Rat (pit and rec) and human FSH are very different. Human recFSH bound to both calf testicular membranes and CHO cells expressing the human FSH receptor (CHO hFSH-R) with about 10-fold higher affinity (Ka) than pituitary and recombinant rat FSH. In in vitro bioassays with immature rat Sertoli cells and CHO hFSH-R cells human recFSH was also about 10-fold more potent than the rat FSH preparations. In the in vitro bioassays with immature rat granulosa cells the difference was about 5-10-fold. These studies indicate that the receptor binding and in vitro activities of rat pitFSH and rat recFSH are similar. The differences in in vivo activity are probably due to the differences in glycosylation. The biological behaviour of rat FSH (pit and rec) is different from that of human FSH. Therefore, if the rat is used as a model for physiology of gonadotrophic action, the results may be greatly influenced by the type (species) of hormone preparation used. The availability of homologous hormone preparations is therefore crucial.


http://www.sciencedirect.com/science/article/B6T3G-45V716K-
The exocrine pancreatic cell line AR42J is also known to display some neuroendocrine (NE) features. We have extended this fact by showing that AR42J cells express mRNA of chromogranin A (CgA), display immunoreactivity (IR) to CgA, and secrete its cleavage product pancreastatin. A sparse occurrence of typical NE secretion granules, together with only a faint IR to conventional NE markers, indicates that the NE cells are of a poorly differentiated type. CgA promoter reporter plasmid experiments showed that gastrin, epidermal growth factor, and phorbol 12-myristate 13-acetate, induce upregulation of CgA after 24 h. By RT-PCR, it was found that AR42J expresses all of the five subtypes of the somatostatin (SST) receptor (SSTR) family, except SSTR4. The existence of functional SSTRs was confirmed by showing that the SST analog octreotide could inhibit gastrin-induced proliferation. Thus, the AR42J cell line may function as a valuable experimental model to study the regulation of CgA and SSTRs in poorly differentiated NE tumor cells.


http://www.sciencedirect.com/science/article/B6T3G-3S0DFD5-8/2/96dd212255b4864156bd06da63cc68a7

Gonadotropin-releasing hormone (GnRH) has been reported to exist in extrahypothalamic tissues such as the placenta, gonads and mammary glands. While we have reported the presence of GnRH-mRNA in the rodent uterus, there have been no reports concerning gene expression of GnRH and its receptor (GnRH-R) in human endometrial tissue. In order to investigate the role of GnRH as a local regulator in the human endometrium, we examined the gene for GnRH and GnRH-R in non-pregnant endometrium and decidua of early pregnancy. Using reverse transcriptase-polymerase chain reaction (RT-PCR) and Southern blot analysis we found GnRH-mRNA but not GnRH-R-mRNA transcripts in the human endometrium and decidua at 7-9 weeks gestation. This is the first report that suggests GnRH gene expression in the human endometrium/decidua.


http://www.sciencedirect.com/science/article/B6T3G-47NWG0N-9K/2/f6720eacf0c9e1756cb77eb884c5a1de

We have localized four transcription initiation sites in the human insulin-like growth factor-I (IGF-I) gene. Two transcription start sites were identified which result in a longer and shorter version of the leader derived from the known exon 1 of the IGF-I gene. Transcription starting at the upstream transcription initiation site results in a leader exon 1 of about 1155 nucleotides (nt), whereas transcription starting at the downstream initiation site results in a leader of about 240 nt. The majority of the transcripts initiate at the latter site. We further identified a region in the human IGF-I gene between exons 1 and 2, which shows a high degree of homology with the rat IGF-I leader exon 1B. By means of the polymerase chain reaction (PCR) we detected human IGF-I mRNAs containing this novel leader. The corresponding exon was designated exon 1B according to the rat IGF-I gene terminology. PCR and RNase protection analyses identified two transcription start sites within this alternative leader exon 1B. Transcription initiated at the most upstream start site results in a leader of about 750 nt, whereas transcription starting at the downstream site is
heterogeneous, resulting in leaders of 65-75 nt long. No consensus TATA-box or AT-rich regions are present immediately upstream of all four transcription start sites identified, nor are these regions particularly GC-rich. The IGF-I gene is known to be expressed differentially in a tissue- and development-specific fashion. Differential activation of multiple promoters could very well play a crucial role in IGF-I gene regulation.


Although evidences are emerging that dietary isoflavones have beneficial effects in treatment of hyperlipidemia and cardiovascular diseases, the underlying molecular mechanism has not yet been extensively characterized. In this report, we showed that genistein, one of the major isoflavones, increased expression of genes involved in lipid catabolism such as carnitine palmitoyltransferase 1, liver form (CPT1L) in HepG2 cells, when assayed by real-time reverse-transcriptase polymerase chain reactions as well as Western blotting analysis. The increase in mRNA-level of CPT1L after genistein treatment was not changed in the presence of ICI182780, a potent inhibitor of estrogen receptor, suggesting that this effect of genistein was estrogen receptor-independent. Since these genes involved in fatty acid catabolism are considered putative downstream target genes of peroxisome proliferators-activated receptor [alpha] (PPAR[alpha]), we examined whether expression of PPAR[alpha] was modulated by genistein treatment. Interestingly, genistein induced expression of PPAR[alpha] at both mRNA- and protein-level. Further, genistein activated transcriptional activity of PPAR[alpha], when determined by reporter gene analysis, suggesting genistein as a potential ligand for PPAR[alpha]. Taken together, this study provides a picture of the regulatory action of genistein, as an activator of PPAR[alpha] in fatty acid catabolism and potential use of genistein as lipid-lowering agent.


Vascular endothelial growth factor (VEGF) plays an essential role in angiogenesis in the growth plate and ultimately in regulating endochondral ossification. Since longitudinal bone growth is often disturbed in children who are treated with glucocorticoids, we investigated the effects of dexamethasone on VEGF expression by epihyseal chondrocytes. Cells were cultured from tibial growth plates of neonatal piglets. Using Northern blotting and RT-PCR techniques, the chondrocyte-specific markers aggrecan, collagen II and CD-RAP were detected. Also the glucocorticoid receptor (GR) was expressed. VEGF protein secreted from these cells was examined by ELISA and Western immunoblotting. The VEGF121 and VEGF165 isoforms were detected in the supernatant. As determined by RT-PCR, all three major mRNA splice variants were produced, including the species encoding VEGF189. Dexamethasone (100 nM) inhibited both protein and mRNA expression by approximately 45%. Hydrocortisone (cortisol) and prednisolone also inhibited VEGF secretion, but they were less active than dexamethasone. The inhibitory actions of dexamethasone were almost completely blocked by the GR antagonist Org34116, indicating that the GR mediates these actions. Degradation of the VEGF mRNA was not accelerated by dexamethasone. Therefore, a transcriptional mechanism seems likely. Downregulation of this important growth factor could lead to disruption of the normal invasion of
blood vessels in the growth plate, which could contribute to disturbed endochondral ossification and growth.


http://www.sciencedirect.com/science/article/B6T3G-47NWGNKJP/2/bfaf52e828405223ab0ccf311aea2ba2

An alternatively spliced mRNA coding for a variant estrogen receptor (ER) missing exon 4 (ER [Delta]4) was detected in the breast tumor cell line MCF7 and meningioma tissue by using the reversed transcriptase PCR technique. The trans-activational properties of this mutant ER were assessed in embryo carcinoma P19EC and human choriocarcinoma JEG3 cells by co-transfection of the ER [Delta]4 expression vector with an oxytocin promoter construct containing an estrogen-responsive element. ER [Delta]4 did not trans-activate the oxytocin promoter in either a hormone-dependent or -independent manner. Co-transfection of ER [Delta]4 together with the wtER did not show any interference of ER /gDA4 on the stimulation of the oxytocin promoter by the wtER. ER [Delta]4 was translated in vitro. Its capacity to bind estradiol, and the binding of the variant to a synthetic estrogen-responsive element were compared to those of the wild-type receptor. ER [Delta]4 did not bind to a synthetic estrogen-responsive element, nor did it bind estradiol. Hence, ER [Delta]4 appears to be a silent variant and we speculate that it is without any role in tumor progression.


http://www.sciencedirect.com/science/article/B6T3G-47MCKW8TX/2/ddf86b897f940cc8bc6e67b966a5fe39

Calbindin-D9k (CaBP-9k) is a calcium binding protein expressed at high levels in the rat uterus. The CaBP-9k gene carries an estrogen response element which is involved in the steroid hormone regulation of the gene during the estrous cycle and gestation. The present study was aimed at determining expression of the gene during the first half of pregnancy and to assess the role of progesterone (P4) and the estrogen receptor (ER). Expression of CaBP-9k mRNA was determined by Northern blot analysis during the first 10 days of pregnancy. On pregnancy day 1 (P1), CaBP-9k mRNA levels were relatively high. On P2, 3 and 5 CaBP-9k mRNA decreased to the detection limit using 10 [mu]g total RNA probed with a random primed cDNA. On P10, CaBP-9k transcripts began to reappear at levels of about 30% of P1. Expression of [beta]-actin mRNA displayed a continuous increase during this period with a rapid rise of 240% between P2 and P3. The typical increase of P4 accompanied by moderate changes of estradiol (E2) was determined in serum of experimental groups. When RU 486 at 10 mg/kg was administered as a single s.c. injection on P3, the CaBP-9k down-regulation was rapidly interrupted and mRNA expression became extremely high. The effect was seen maximally at 24 h post injection and was maintained at 48 and 72 h. Expression of [beta]-actin mRNA was increased only moderately at 24 h and was unchanged at 48 and 72 h. Serum P4 remained unaffected by the treatment and E2 displayed a slight increase. When ER mRNA was quantified by reverse transcription/PCR techniques a more than 300% increase was detected in the RU 486 treated rats. These data implicate that P4 in early pregnancy is responsible for down regulation of the CaBP-9k gene in uterus via an indirect effect involving the expression of the estrogen receptor.

http://www.sciencedirect.com/science/article/B6T3G-46HBR4P-1/2/d5f55fa3e8600c50a139216cecb7b947

The trout glucocorticoid receptor (rtGR) contains an additional sequence of nine amino acids located between the two zinc fingers of the DNA-binding domain (DBD) (Endocrinology 136 (1995) 3774). Polymerase chain reaction on trout genomic DNA and sequencing were performed in the DBD region, demonstrating that this peptide is encoded by an additional exon of 27 nucleotides between the two exons encoding the two zinc fingers of other nuclear receptors. This additional sequence in the rtGR confers a better binding affinity of the receptor to a single GRE, as shown by gel shift experiments with GST-DDBtGR fusion proteins, deleted or not of the nine amino acids ([Delta][9]). This higher affinity is correlated with a higher constitutive transcriptional activity of the receptor on a reporter gene driven by a single GRE, but not with the ligand-induced transcriptional activity. Nevertheless, on a double GRE, the wild type and rtGR-[Delta][9] are equally active on both constitutive or dexamethasone-induced transcriptional activity. This original DBD structure could have emerged during evolution such as to allow better regulation of glucocorticoid dependent genes in relation to the large spectrum of cortisol physiological functions in fish.


http://www.sciencedirect.com/science/article/B6T3G-4CDS5V6-2/2/665db4b9a84f85b9b341edb60a80fe6f

Vitamin D 1[alpha]-hydroxylase (1[alpha](OH)ase), which converts the circulating prohormone 25-hydroxyvitamin-D3 (25(OH)D3) to the active 1[alpha]-25-dihydroxyvitamin-D3 (1,25(OH)2D3), is present in normal prostatic epithelium. However, prostate cancer cells, both primary cultured cells and cell lines, have greatly decreased activity of 1[alpha](OH)ase and are therefore resistant to the tumor suppressor activity of circulating 25(OH)D3. We quantitated 1[alpha](OH)ase mRNA and protein levels to investigate mechanism(s) responsible for decreased 1[alpha](OH)ase enzymatic activity in prostate cancer. Prostate cancer cell lines had low 1[alpha](OH)ase mRNA levels. Primary prostate cell cultures derived from normal and cancer tissues had equivalent levels of 1[alpha](OH)ase RNA and protein. Equivalent 1[alpha](OH)ase protein levels were observed in prostate tissue sections containing normal and malignant cells. The protein levels of hsc70, whose homolog intracellular Vitamin D binding protein (IDBP-1) facilitates delivery of 25(OH)D3 to 1[alpha](OH)ase in monkey cells, were equivalent in the normal and cancer cells. Equivalent activity in normal and cancer cells of Vitamin D 24-hydroxylase, a mitochondrial enzyme that also uses 25(OH)D3 as a substrate, further ruled out lack of access to substrate as a basis for low activity of 1[alpha](OH)ase in cancer cells. We conclude that diminished 1[alpha](OH)ase activity in prostate cancer cell lines is through decreased gene expression, whereas decreased activity in primary cultures and tissues is post-translational.

Breast cancer patients with an estrogen receptor (ER) positive tumor can be treated with the anti-
estrogen tamoxifen, but development of anti-estrogen resistance is a serious problem. We have
analyzed a tamoxifen resistant human breast cancer cell line MCF-7/TAMR-1 for alterations in ER
which might explain the tamoxifen resistance. The MCF-7/TAMR-1 cells expressed both wildtype
ER mRNA and protein, and by RT-PCR we were able to clone ER cDNAs corresponding to the
double splice variant lacking both exon 4 and 7 (ER[Delta]E4,7). The existence of the
ER[Delta]E4,7 variant was confirmed by RNase protection assay. Semi-quantitative RT-PCR
revealed that ER[Delta]E2 mRNA was expressed at a higher level in MCF-7/TAMR-1 cells,
whereas the ER[Delta]E5 mRNA was expressed at a significantly lower level in MCF-7/TAMR-1
cells compared with MCF-7 cells. The differential expression of the two ER mRNA splice variants
indicates that they may be involved in anti-estrogen resistance, although the present knowledge
of their biological function does not provide us with an explanation.


Thyroid transcription factor-1 (TTF-1), a tissue-specific nuclear transcription factor involved in the
embryogenesis and differentiation of human thyroid, lung and brain, has been recently identified
in other rat tissues, including parafollicular C cells and parathyroid chief cells. Based on this
distribution, a possible role for this factor in calcium homeostasis has been suggested. This study
investigated the presence of TTF-1 transcripts and protein in human tissues expressing the
calcium sensing receptor (CaSR). Using a RT-PCR technique, complemented by Southern blot
analysis, TTF-1 expression was detected in human C cells (two medullary thyroid carcinomas),
but not in normal and adenomatous (four adenomas and three hyperplasia) parathyroid, and
normal and adenomatous (six adenomas) pituitary tissues. CaSR was expressed in all samples.
The absence of expression was confirmed by Western blot. In contrast to previous studies in the
rat, this study demonstrates the absence of TTF-1 transcripts in the human adult parathyroid and
pituitary glands, although a role for this factor during the ontogeny of these organs cannot be
excluded.

Miki, Y., T. Suzuki, et al. (2005). "Steroid and xenobiotic receptor (SXR), cytochrome P450 3A4 and
multidrug resistance gene 1 in human adult and fetal tissues." Molecular and Cellular
Endocrinology 231(1-2): 75.

The steroid and xenobiotic receptor (SXR) has been demonstrated to play an important role in the
regulation of the cytochrome P450 3A4 gene (CYP3A4) and multidrug resistance gene 1 (MDR1)
by both endogenous and xenobiotic substrates. SXR and its rodent ortholog PXR exhibit marked
differences in their ability to be activated by xenobiotic inducers. This suggests that results
obtained by rodent models may not always accurately predict responses to the same compounds
in humans. SXR expression was demonstrated in the human liver and intestine, but its systemic
distribution remains unknown. Therefore in this study, we first characterized the expression of
SXR and its target genes CYP3A4, and MDR1 in human adult and fetal tissues using quantitative RT-PCR, immunoblotting, and combined laser capture microscopy and RT-PCR analysis. SXR mRNA and protein are expressed in adult and fetal liver, lung, kidney, and intestine. There is a close association between the expression of SXR and its target genes in all of the tissues examined. The amounts of SXR mRNA in the liver and intestine reached maximal levels in young adults (15-38 years old) and then subsequently decreased to less than half of the maximal levels with aging. These findings demonstrated age-related differences in the body's capacity to metabolize steroids and xenobiotic compounds and suggest an important role for SXR and its target genes, CYP3A4 and MDR1 in this process.


http://www.sciencedirect.com/science/article/B6T3G-47MCJDT-50/2/f59d95f4dd3cd08bdd07c320e55251a4

A segment of DNA was amplified from the Neurospora crassa genome by the polymerase chain reaction using several oligonucleotides coding for highly conserved domains in proinsulin as primers and probe. A genomic clone corresponding to this segment was isolated and the nucleotide sequence was determined. The deduced amino acid sequence of a part of this segment bears remarkable resemblance to preproinsulin, but lacks several requirements for transcription or translation and must therefore be considered to be a pseudogene.


http://www.sciencedirect.com/science/article/B6T3G-4BBHDY4-7/2/05a261e86d2a04511fcf29425a720bce

Background: In patients with glucocorticoid remediable aldosteronism (GRA), a rare hypertensive disorder caused by the presence of a chimeric aldosterone synthase (CYP11B2) and 11[beta]-hydroxylase (CYP11B1) gene, high level of urinary 18-hydroxycortisol (18OHF) excretion are observed. In some patients with hypertension, increased urinary 18OHF secretion is also found in the absence of the hybrid CYP11B1/CYP11B2 gene. We hypothesised that gene variants of CYP11B1 or CYP11B2 may be linked to this abnormal glucocorticoid production. Methods: The urinary steroid profile was analysed by gas chromatography/mass spectrometry in 429 hypertensive patients and 98 (23%) thereof tested positive for increased 18OHF excretion. After correction for total cortisol excretion, 12 subjects showed an abnormally high 18OHF excretion. For genotyping DNA was obtained from six of these patients. All were tested negative for the hybrid CYP11B1/CYP11B2 gene and were further analysed for mutations in all exons and promoter regions of both CYP11B1 and CYP11B2 by single strand conformation polymorphism (SSCP) and sequencing when appropriate. Results: The genetic analysis of the two genes revealed the presence of nine molecular variants in CYP11B2 and three in CYP11B1. In addition to published polymorphic sites, we identified two new variants in CYP11B2 but no new variants in CYP11B1. The newly identified CYP11B2 mutations are a C/T single nucleotide exchange located in the first intron and a double nucleotide exchange at the 3'-splice site of exon 8. The mutated sequence corresponds to the sequence of CYP11B1 indicating a gene conversion. This suggests that the mutant is not likely to affect splicing. Thus, none of the genetic variants identified explains the high urinary excretion of 18OHF. Conclusions: We present here a complete method for the genetic analysis of the CYP11B1 and CYP11B2 genes. By this method we could not identify genetic variants responsible for a GRA-like phenotype. The presence of high levels of 18OHF should not be used alone as a diagnosis tool for GRA.
The majority of ovarian tumors are derived from the single layer of epithelial cells on the surface of the ovary termed the ovarian surface epithelium (OSE). Stromal cell-OSE interactions are postulated to be an important aspect of normal OSE biology and the biology of ovarian cancer. Transforming growth factor beta (TGF[beta]) has been shown to often be a mesenchymal cell-derived growth factor that mediates stromal cell-epithelial cell interactions in a variety of different tissues. The current study investigates the expression and action of TGF[beta] isoforms (TGF[beta]1, TGF[beta]2, and TGF[beta]3) in OSE and the underlying stroma in both normal bovine and human tumor tissues. Normal bovine ovaries are similar to human oocytes and are used as a model system to investigate normal OSE and stromal cell functions. All three TGF[beta] isoforms and their receptor, transforming growth factor beta receptor type II (TGF[beta]RII), proteins were found to be detected in the OSE from normal bovine ovaries using immunohistochemistry. Ovarian stromal tissue also contained positive immunostaining for TGF[beta] isoforms and TGF[beta]RII. RNA was collected from normal bovine OSE and ovarian stromal cells to examine TGF[beta] gene expression. TGF[beta]1, TGF[beta]2, and TGF[beta]3 transcripts were detected in both freshly isolated and cultured bovine OSE and stromal cells by a sensitive quantitative polymerase chain reaction assay. TGF[beta]1 and TGF[beta]2 mRNA levels were found to be present at similar levels in freshly isolated OSE and stroma. Interestingly, TGF[beta]3 mRNA levels were significantly higher in freshly isolated OSE than stromal cells. All but TGF[beta]3 mRNA in OSE increased when the cells were cultured. Observations indicate that normal bovine OSE and stroma cells express the three TGF[beta] isoforms in vivo and in vitro. Human ovarian tumors from stage II, stage III and stage IV cases were found to express TGF[beta]1, TGF[beta]2, TGF[beta]3 and TGF[beta]RII protein primarily in the epithelial cell component by immunohistochemistry analysis. The stromal cell component of the human ovarian tumors contained little or no TGF[beta] or TGF[beta]RII immunostaining. TGF[beta] actions on bovine OSE and stromal cells were also investigated. TGF[beta] was found to inhibit the growth of OSE, but not stromal cells. To further examine the actions of TGF[beta] on OSE, the expression of two growth factors previously shown to be expressed by OSE were analyzed. TGF[beta]1 was found to stimulate the expression of both keratinocyte growth factor (KGF) and kit ligand/stem cell factor (KL) by bovine OSE. Therefore, TGF[beta] actions on OSE will likely promote a cascade of cell-cell interactions and cellular responses involving multiple growth factors. The effects of regulatory agents on TGF[beta] expression by the bovine OSE were examined. Transforming growth factor alpha (TGF[alpha]) stimulated TGF[beta]1 expression, TGF[beta]1 stimulated TGF[beta]2 expression, and follicle stimulating hormone (FSH) stimulated TGF[beta]3 expression. These results demonstrate that TGF[beta] isoforms are regulated differently by the regulatory agents tested. In summary, all the TGF[beta] isoforms are differentially expressed by the OSE and TGF[beta] appears to have an important role in regulating OSE and possibly stromal-OSE interactions. A complex network of endocrine and paracrine interactions appears to influence the expression and actions of TGF[beta] on OSE. Abnormal expression and/or action of TGF[beta] is postulated to in part be involved in the onset and progression of ovarian cancer.
In a sexually mature female, primordial follicles continuously leave the arrested pool and undergo the primordial to primary follicle transition. The oocytes increase in size and the surrounding squamous pre-granulosa cells become cuboidal and proliferate to form a layer of cuboidal cells around the growing oocyte. This development of the primordial follicle commits the follicle to undergo the process of folliculogenesis. When the available pool of primordial follicles is depleted reproductive function ceases and humans enter menopause. The current study examines whether leukemia inhibitory factor (LIF) promotes the primordial to primary follicle transition that initiates follicular development. Ovaries from 4 day-old rats were cultured in the absence or presence of LIF or neutralizing antibody to LIF. LIF treatment increased the proportion of follicles that initiated the primordial to primary follicle transition to 59%, compared to 45% in untreated cultured ovaries. The ability of LIF to induce primordial follicle development was enhanced to greater than 75% by the presence of insulin in the culture medium. Anti-LIF neutralizing antibody reduced the proportion of spontaneous developing primordial follicles. Immunocytochemical studies demonstrated higher levels of LIF protein in the granulosa and surrounding somatic cells of primordial and primary follicles compared to the oocyte. In contrast, later pre-antral and antral stage follicles showed LIF expression primarily in the oocyte. In granulosa and theca cell cultures LIF had no effect on cell proliferation. However, LIF treatment did increase expression of Kit ligand (KL) mRNA in cultured granulosa cells. KL has been shown to promote ovarian cell growth and induce primordial follicle development. LIF induction of KL expression may be involved in the actions of LIF to promote primordial to primary follicle transition. In summary, LIF treatment increased the primordial to primary follicle transition in cultured ovaries and LIF may interact with KL to promote primordial follicle development.


http://www.sciencedirect.com/science/article/B6T3G-4BDM4B2-7/2/e91c96ad2af742c2a4c6aa16f7046f49

Ovulated eggs during a female's reproductive life are derived from a pool of primordial follicles arrested in prophase of the first meiotic division. When follicles leave the resting pool they undergo a primordial to primary follicle transition and will grow and develop until either ovulation occurs or follicles undergo atresia. Several growth factors have been implicated as acting locally within the ovary to regulate the primordial to primary follicle transition. How these growth factors may interact and cooperate to perform this vital function remains to be elucidated. The objective of the current study is to investigate interactions between kit ligand (KL) (i.e. stem cell factor) and basic fibroblast growth factor (bFGF) that promote the primordial to primary follicle transition in rat ovaries. Ovaries were removed from 4-day-old rat pups and cultured for 2 weeks with KL alone or with KL and a neutralizing antibody against bFGF. The ability of KL treatment to increase primordial follicle transition was blocked with a bFGF neutralizing antibody. In addition, ovary cultures were treated with bFGF alone or with bFGF and an anti-c-kit receptor antibody which blocks KL signaling. The ability of bFGF treatment to increase primordial follicle transition was blocked with an anti-c-kit receptor antibody. Observations indicate that both KL and bFGF must be active in order to optimally promote the changes that occur in oocytes, granulosa cells, and stromal/interstitial cells when primordial follicles initiate development. Cultured ovaries were treated with either KL or bFGF for 3 days and then bFGF and KL mRNA expression levels in the whole ovary were measured. KL was not found to regulate bFGF expression. In contrast, bFGF treatment was found to increase KL mRNA expression in cultured ovaries. These observations suggest that one function of the oocyte-derived bFGF is to increase the granulosa derived KL expression and that both KL and bFGF are required to optimally promote primordial to primary follicle transition. Elucidating the cell-cell interactions that mediate this network of specific locally
derived growth factors is critical to understanding the physiology of the primordial to primary follicle transition.


Limited information is available concerning the regulation of growth hormone-releasing hormone (GHRH) gene expression in the hypothalamus, largely because of the lack of a suitable cellular model. In an attempt to immortalize hypothalamic GHRH-producing neurons, we have generated a transgenic mouse model which expresses the simian virus 40 (SV40) T-antigen gene (Tag) under the control of the GHRH gene promoter. The transgene contains ap 5 kb of mouse GHRH gene sequences, including 3.5 kb of the 5'-flanking region, the entire hypothalamic exon 1 and 1.5 kb of intron 1, fused to the SV40 Tag gene. This construct was microinjected into fertilized oocytes. Fourteen of 96 mice born had integrated the transgene. These mice were fertile and showed no signs of central or peripheral tumors. The pattern of expression of the SV40 Tag gene was analyzed in four different transgenic lines by RT-PCR. The tissues tested include: hypothalamus, pituitary, cortex, cerebellum, spinal cord, adrenal, testis, spleen and lung. Transgene expression was consistently detected in the hypothalamus of all lines. In addition, SV40 Tag expression was also detected in the hypothalamus by Northern blot analysis in two of the transgenic lines. SV40 Tag expression was also detected in the testis of all transgenic lines by RT-PCR. This result was not expected since the GHRH gene sequences present in the transgene do not include the testis-specific transcription initiation site previously described. This suggests that GHRH gene expression in the mouse testis can be directed by regulatory sequences located downstream of the testis specific transcription start site. We conclude that the promoter region of the GHRH gene included in this construct contains the regulatory elements necessary to drive hypothalamic and testis expression in vivo. In addition, all mice from one of the transgenic lines developed cataracts in both eyes. SV40 Tag expression was detected not only in eyes with cataracts, but also, to a lesser extent, in eyes from other transgenic lines. Furthermore, the endogenous GHRH gene was found to be expressed in the eyes of normal mice.


It has been reported that LH receptor (LHR) mRNA is not detected in cumulus cells of porcine cumulus-oocyte complexes (COCs) just after collection from small antral follicles. The present study showed that the formation of LHR in cumulus cells was up-regulated by the cultivation with 20 ng/ml FSH. When the newly synthesized receptors were stimulated by 1.0 [mu]g/ml LH, significantly higher levels of cAMP and progesterone production in cumulus cells were observed as compared with those of COCs cultured with FSH. A loss of proliferative activity of cumulus cells was induced by the additional LH to FSH-containing medium; however, the inhibitory effect was overcome by progesterone receptor antagonist RU486. Furthermore, the addition of LH also accelerated ongoing GVBD in cumulus cells-enclosed oocytes. These results revealed that during in vitro meiotic maturation of porcine COCs, progesterone secreted by FSH- and LH-stimulated cumulus cells reduced proliferative activity of cumulus cells; the changes of cumulus cells might be involved in inducing meiotic resumption of porcine oocytes.

http://www.sciencedirect.com/science/article/B6T3G-4BHSPV-5/2/7320a52c8d11166f511f6aa8a62e4c6b

We investigated the expression of heparin-binding epidermal growth factor-like growth factor (HB-EGF) and its receptors in the rat ovary to define the role of HB-EGF in the ovarian function. The expression pattern of HB-EGF mRNA and protein were studied by semi-quantitative RT-PCR and immuno-histochemistry using an antibody that was specifically stained for the precursor form of HB-EGF in naturally cycling rats and immature pseudo-pregnant rat models. The immuno-histochemical study showed that in naturally cycling rats, HB-EGF was expressed in most granulosa cells of early follicles and all the developing follicles but not in preovulatory follicles. This was supported by the semi-quantitative RT-PCR results in that the lowest level of HB-EGF mRNA during the estrous cycle was found in the evening of proestrus when the HB-EGF negative preovulatory follicles were most prominent. The results suggest that HB-EGF might be a mitogen for granulosa cells and down regulation of its expression may be necessary for the final maturation of follicles. In corpora lutea, luteal cells of older generation stained stronger than those of younger generation. Moreover, luteal cells of late luteal phase stained stronger than those of the mid and early luteal phases in the immature pseudo-pregnant rat models, indicating that the precursor form may be associated with death of luteal cells. Finally, of the two cognate receptors for HB-EGF, erbB1 was expressed in the rat ovary, but erbB4 was specifically not expressed in this organ. The spatial and temporal pattern of HB-EGF expression suggest that HB-EGF may an important local regulator of ovarian function and structure.


http://www.sciencedirect.com/science/article/B6T3G-427JWGY-1W/2/019fab2cefdb0e017de6b393c2921817

The ability of gonadotropins to act on and regulate normal ovarian surface epithelial (OSE) cells and cancer cells was investigated. Bovine OSE was used as a model to study normal OSE. Results demonstrate that follicle stimulating hormone (FSH) and the luteinizing hormone (LH) like molecule, human chorionic gonadotropin (hCG), can both stimulate (3H)-thymidine incorporation into DNA in normal OSE cells. Similar results were obtained using either purified hormones or recombinant human hormones. A human ovarian cancer cell-line OCC1 was also stimulated to grow in response to FSH and hCG, but the growth of a different human ovarian cancer cell-line SKOV3 was not affected. In addition to effects on cell growth, gonadotropins also stimulated growth factor expression. Both FSH and hCG stimulated steady state levels of keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), and kit ligand (KL) mRNA in OSE cells. Previously, KGF, HGF, and KL have been shown to stimulate OSE growth. Both follicle stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) were observed in OSE cells by Northern blot analysis. Reverse transcription polymerase chain reaction (RT-PCR) analysis was performed on fresh and cultured OSE cells. Normal OSE was found to express FSHR and LHR both in vivo and in vitro. The PCR reaction products were sequenced and found to provide a 100% homology with the bovine gonadotropin receptor sequences previously reported. FSHR and LHR transcripts were also detected in gonadotropin responsive OCC1 cells, but not in the gonadotropin insensitive SKOV3 cells. Observations support the hypothesis that gonadotropins may influence some ovarian cancers. In summary, the current study demonstrates the novel observation that both the FSHR and LHR are expressed by bovine...
OSE and selected ovarian cancers. Interestingly, the actions of FSH and LH to promote OSE growth may in part be mediated indirectly through an elevation in the expression of autocrine growth factors (KGF, HGF, and KL). Ovarian cancer is more common in conditions with elevated gonadotropins such as post-menopausal women. Therefore, gonadotropin actions on the OSE are postulated to be a potential factor in the onset and progression of some ovarian cancers.


http://www.sciencedirect.com/science/article/B6T3G-417ND93-8/2/7f5c9c567b7ee804149815b5eaf57aa9

The current study investigates the expression and action of keratinocyte growth factor (KGF) in normal ovarian surface epithelium (OSE) and ovarian cancer tissues. Ovarian tumors are primarily derived from the OSE. KGF is a mesenchymal cell-derived growth factor that mediates stromal cell-epithelial cell interactions in a variety of different tissues. Human ovarian tumors from borderline, stage I and stage III cases were found to express KGF protein in the epithelial cell component by immunocytochemical analysis. The stromal cell component of human ovarian tumors contained little or no KGF immunostaining. Normal bovine ovaries have similarities to human ovaries and are used as a model system to investigate normal OSE functions. KGF protein was detected in the OSE from normal human and bovine ovaries by immunocytochemistry. Ovarian stromal tissue contained light but positive KGF immunostaining. RNA was collected from normal bovine OSE and ovarian stromal cells to examine KGF gene expression. KGF transcripts were detected in cultured OSE and stromal cells by Northern blot analysis. In order to examine and quantitate KGF gene expression in freshly isolated versus cultured tissues, a sensitive quantitative RT-PCR assay for KGF was utilized. KGF gene expression was found to be high in freshly isolated OSE, but very low in freshly isolated stroma. Levels of KGF gene expression after culture of OSE and stromal cells increased. Observations indicate that normal OSE express high levels of KGF in vivo and in vitro. Expression of KGF by normal epithelial cells versus stromal cells was unexpected and suggests KGF may be an important autocrine stimulator of OSE. KGF actions on bovine OSE cells were investigated. KGF was found to stimulate the growth of normal OSE cells to the same level as epidermal growth factor. Two ovarian cancer cell lines, SKOV3 and OCC1, were also stimulated to proliferate in response to KGF. Current results demonstrate the production and action of KGF on normal OSE cells and ovarian cancer cells. Observations can be interpreted to suggest that KGF may in part be involved in the growth of ovarian tumors. This appears to be one of the first reports of KGF production by an epithelial cell. The autocrine stimulation of OSE growth by the local production and action of KGF provides insight into how the OSE may develop abnormal growth characteristics involved in the onset and progression of ovarian cancer.


http://www.sciencedirect.com/science/article/B6T3G-3RSN3V8-2/2/95396995e11007bcd62a80b11c2ea2d2

By the application of RT-PCR, we have demonstrated that in the human endometrium mRNAs for insulin-like growth factors, IGF-I and II, and their receptors are expressed not only in the intact endometrium, but also in the freshly isolated stromal and epithelial cells. The expression of multiple transcript forms of the IGF-I and II at various phases of the menstrual cycle, occurs by differential use of all four IGF-I transcriptional start sites, and two of the four known promoter sites
of the IGF-II gene. The complete spectrum of transcripts is displayed by the proliferative phase and the menstrual phase endometrium. During the secretory phase, the exon 1 upstream start site of the IGF-I gene and the P2 promoter of the IGF-II gene are not used. Irrespective of the phase of the menstrual cycle, the stromal cells always display the same transcriptional patterns of both growth factor genes as those of the intact endometrium. In contrast, the epithelial cells do not express IGF-I transcript originating from the exon 2 upstream initiation site. These results indicate that the expressions of the IGF-I and II genes in the intact endometrium and stromal and epithelial cells are modulated at the transcriptional level during the menstrual cycle by differential usage of promoters and start sites.


http://www.sciencedirect.com/science/article/B6T3G-3WWDH35-3/2/a4b6e34802c7bd57fd54c69d3c06f604

The inappropriate expressions of insulin-like growth factors (IGF-I and II) and IGF-I receptor (IGF-IR) are implicated in the malignant growth of many cancers. To determine changes, if any, in the levels of expression of IGFs and IGF receptor genes in neoplastic endometrium, relative to normal endometrium, the mRNA levels of IGF-I and II and of IGF-IR and IIR were measured in samples of endometrial carcinomas (EC) and normal endometrium, through all phases of the menstrual cycle, by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) assays. In normal endometrium, the mRNA levels of IGF-I were elevated in the proliferative and early secretory phases. The IGF-II mRNAs were relatively high in the proliferative phase, but unaltered through early and late secretory phases. Significantly elevated levels of IGF-II transcripts were observed during the menstrual phase, suggesting a possible role of IGF-II in endometrial regeneration. A positive correlation between the levels of IGF-I and IGF-IR mRNAs, apparent in the samples of normal endometrium, was not observed in endometrial carcinomas. The IGF-IR and IIR mRNA levels were elevated in endometrial carcinoma samples. On the other hand, the IGF-I and II mRNA levels were conspicuously low in many carcinoma samples, which were not associated with hyperplasia (type II EC), but relatively elevated in two other carcinoma samples, associated with adenomatous hyperplasia (type I EC). These results albeit with few samples suggest the possibility that the overexpressed receptor, IGF-IR, could be activated differently in two types of endometrial carcinomas, namely ligand-dependently in type I ECs and ligand-independently in type II ECs.


http://www.sciencedirect.com/science/article/B6T3G-40XWF2-V/2/fe6c5cbf22279fd98a755e70b656acb

The steroid aldosterone plays a major role in the maintenance of total body sodium homeostasis and also contributes to cardiovascular pathophysiology by mediating cardiac hypertrophy and fibrosis. In addition to classical adrenal production of aldosterone, endogenous tissue production of aldosterone has been observed in various organs; aldosterone biosynthesis in cardiac tissues, however, remains highly controversial. The current study provides a comprehensive evaluation of steroid hormone biosynthetic capabilities in multiple tissues from two distinct rat strains under unstimulated and stimulated conditions. Panels of tissues from Wistar and Sprague-Dawley rats were probed for 11 [beta] hydroxylase (P45011[beta]) and aldosterone synthase (P450aldo) by reverse transcriptase-polymerase chain reaction (RT-PCR). Under unstimulated conditions,
cardiac P45011[β] and P450aldo were detected only in Wistar rats. Angiotensin II (100 μg/day) stimulated myocardial expression of both enzymes in both strains. Cerebral cortex and mesenteric artery message levels in both strains was reduced by angiotensin II. These data demonstrate the potential for local steroid synthesis in vascular, cardiac, renal, and neuronal tissues, and that biosynthesis of non-adrenal aldosterone may be differentially regulated between strains. This variability may thus resolve in part or whole the current controversy over the existence of non-adrenal steroidogenic systems.


http://www.sciencedirect.com/science/article/B6T3G-4BV484D-1/2/e7c0b4fe059dbd9dfc82ef7c70360463

The expression levels of three estrogen receptor (ER) isotypes alpha, beta, and gamma were quantified in female largemouth bass (Micropterus salmoides) (LMB) liver, ovary, brain, and pituitary tissues. ER alpha and beta expression predominated in the liver, while ERs beta and gamma predominated in the other tissues. Temporally in females, ER alpha was highly up-regulated, ER gamma was slightly up-regulated, and ER beta levels remained unchanged in the liver when plasma 17-[β]-estradiol (E2) and vitellogenin (Vtg) levels were elevated in the spring. In ovarian tissue from these same fish, all three ERs were maximally expressed in the fall, during early oocyte development and prior to peak plasma E2 levels. When males were injected with E2, ER alpha was highly inducible, ER gamma was moderately up-regulated, and ER beta levels were not affected. None of the ER isotypes were induced by E2 in gonadal tissues. These results combined suggest that the ERs themselves are not regulated in the same manner by E2, and furthermore, do not contribute equally to the transcriptional regulation of genes involved in fish reproduction such as Vtg.


http://www.sciencedirect.com/science/article/B6T3G-4DW3CTN-1/2/30559f4b88b821ae45af0bd78aa5f09b

Inactivating mutations in the LH receptor are the predominant cause for male pseudohermaphroditism in subjects with Leydig cell hypoplasia (LCH). The severity of the mutations, correlates with residual receptor activities. Here, we detail the clinical presentation of one subject with complete male pseudohermaphroditism and LCH. We identify within the proband and her similarly afflicted sibling a homozygous T to G transversion at nucleotide 1836 in exon 11 of the LH/CGR gene. This causes conversion of a tyrosine codon into a stop codon at codon 612 in the seventh transmembrane domain, resulting in a truncated receptor that lacks a cytoplasmic tail. In vitro, in contrast to cells expressing a normal LHR, cells transfected with the mutant cDNA exhibit neither surface binding of radiolabeled hCG nor cAMP generation. In vitro expression under the control of the LHR signal peptide of either a wild type or mutant LHR-GFP fusion protein shows no differences in receptor cellular localization. In conclusion, the in vitro studies suggest that residues in the seventh transmembrane domain and cytoplasmic tail are important for receptor binding and activation without playing a major role in receptor cellular trafficking.
The prevalence of hypertension and atherosclerosis among subjects with hyperinsulinemia supports the premise of a direct metabolic link between insulin and angiotensin II at the cellular level. In the present study, the effect of insulin on the angiotensin II-induced growth of A10 smooth muscle cells (SMC) was investigated. Treatment of quiescent A10 cells with angiotensin II caused an increase in RNA synthesis, proto-oncogene c-fos mRNA levels and cell size dependent upon pretreatment with insulin. The insulin requirement was independent of its actions as a growth factor, since a pre-treatment of at least 24 h with insulin was essential for growth stimulation by angiotensin II. Using RT-PCR, insulin was shown to regulate AT2 receptor expression in both quiescent and differentiating cells. These data suggest the AT2 receptor, which mediates the growth effects of angiotensin II in A10 cells, may be the critical target for the effect of insulin.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a member of the secretin/glucagon/vasoactive intestinal peptide (VIP)/growth hormone releasing hormone (GHRH) family of neuropeptides, several of which stimulate steroidogenesis in ovarian granulosa cells. PACAP receptors are of two major subtypes; the type I receptor (PACAP-I-R) has much higher affinity for PACAP than VIP, and the type II receptor (PACAP-II-R) has similar affinity for both peptides. In the rat ovary, expression of the PACAP gene was demonstrated by amplification of ovarian RNA by the reverse transcription/polymerase chain reaction (RT-PCR). In addition, hybridization of Northern blots of rat ovarian poly(A)+ RNA with a 706-nt rat hypothalamic PACAP-I-R cDNA probe revealed the presence of a 7.0 kb PACAP receptor transcript, similar to that detected in brain and hypothalamus. RT-PCR using specific primers for the PACAP-I-R gene yielded products of the expected size with RNA obtained from ovarian tissue, brain, and hypothalamus. The authenticity of the PCR products was confirmed by Southern blotting and nested PCR, which revealed at least three splice variants of the PACAP-I-R in the rat ovary. These findings demonstrate that both PACAP and PACAP-I-R isoforms are expressed in the rat ovary, where they could exert autocrine or paracrine actions on granulosa cell function.

Myotonic dystrophy (DM), Huntington's disease (HD) and Fragile X syndrome (FRAXA) are three monogenic disease which are caused by so-called dynamic mutations. These mutations are caused by triplet repeats inside or in the vicinity of the gene which have the tendency to expand beyond the normal range thus disrupting the normal functioning of the gene. We describe here
our experiences from 1995 to May 2000 with PGD for these three triplet repeat diseases.


http://www.sciencedirect.com/science/article/B6T3G-3WM5C4D-G/2/4b4da433827bf8df70281e05366cc66f6

Ablation of pituitary gonadotrophs was obtained in transgenic mice expressing diphtheria toxin A (DTA) under control of the -313/+48 bovine glycoprotein hormone [alpha]-subunit ([alpha]SU) promoter, previously shown to be active in mouse gonadotrophs but not in thyrotrophs. Development of hormone-producing cell types was assessed on the day of birth by computer-assisted image analysis on paraffin-embedded, immunostained pituitary sections. Six out of 50 transgenic F0 ('founder') mice (3 males and 3 females) showed a nearly complete disappearance of gonadotrophs but not of thyrotrophs. The number of lactotrophs and the relative area occupied by PRL-immunoreactivity were significantly reduced in the gonadotroph-depleted mice. The size of lactotroph clusters was smaller in the absence of gonadotrophs. The number and immunoreactive area of corticotrophs and somatotrophs, on the other hand, were not significantly affected by gonadotroph ablation. Based on the reported evidence that fetal ovaries do not produce steroid hormones as a result of lack of expression of at least three of the steroidogenic enzymes, P450scc, P450c17, and P450arom, the present observations can hardly be explained by a decline in estrogen levels due to gonadotroph ablation. Rather, the present data indicate that gonadotrophs directly stimulate the development of lactotrophs during fetal and early postnatal life, consistent with previous in vitro observations, and/or that gonadotrophs may share a cell-lineage relationship with a subpopulation of lactotrophs.


http://www.sciencedirect.com/science/article/B6T3G-4233BNC-D/2/987a54374f43c51de959723e36a9e306

Transcription of the human neutral endopeptidase 24.11 (NEP) gene is androgen regulated in prostate cancer cells. Homology search identified a sequence GTCACAaagAGTTCT similar to the ARE consensus sequence GGTACAnnTGTTCT within the 3'-untranslated region of the NEP mRNA. A double-stranded radiolabelled oligonucleotide containing this NEP-ARE sequence formed a DNA-protein complex with nuclear proteins from LNCaP cells or COS-7 cells co-transfected with an androgen receptor (AR) expression vector, and with full-length AR synthesized by baculovirus in mobility shift assays. Unlabeled NEP-ARE or consensus ARE but not mutated NEP-ARE replaced radiolabelled NEP-ARE. Steroid-dependent enhancement of transcription was assayed by transfecting ptkCAT reporter constructs containing the NEP-ARE into CV-1/AR cells and prostate cancer cells (PC-3/AR). Enhancement of chloramphenicol acetyltransferase (CAT) activity was increased four-fold by androgen, seven-fold by dexamethasone and three-fold by progesterone in CV-1/AR cells, and the NEP-ARE bound to glucocorticoid and progesterone receptor in mobility shift assays. We next performed DNase-I footprinting analysis of the NEP promoter and identified a 23 bp sequence GGTGCGGGTCGGAGGGATGCCCA (NEP-ARR) which was protected from DNase I cleavage by nuclear extracts from COS-7 cells expressing AR. This sequence was 62.5% homologous to an androgen responsive region (PSA-ARR) identified in the promoter of the prostate specific antigen (PSA) gene. A double-stranded radiolabelled oligonucleotide containing this NEP-ARR
sequence formed DNA-protein complex with AR but not GR proteins. Unlabeled NEP-ARR, PSA-ARR and NEP-ARE replaced radiolabelled NEP-ARR. Steroid-dependent enhancement of transcription assays in PC-3/AR cells revealed that the enhancement of CAT activity was increased 2.3-fold by androgen, but not by glucocorticoid or progesterone. In a thymidine kinase promoter, the NEP-ARE and NEP-ARR together stimulated a five-fold increase in promoter activity in PC cells. These data suggest that steroid regulation of the NEP gene involves at least two elements including a typical ARE which binds androgen, progesterone and glucocorticoid receptors, and a unique ARR which only binds androgen receptor.


http://www.sciencedirect.com/science/article/B6T3G-3YS34B7-G/2/c73ceae48348f8645e48ab656ada6b3c

Recent evidence has shown that bone is not only a target of estrogen action but also a source of local estrogen production. Bone cells such as osteoblasts express aromatase (P450arom) and the expression of P450arom in osteoblasts is positively regulated in a tissue specific fashion, as in the case of other tissues which express P450arom. To clarify the physiological factors regulating expression of P450arom in bone, we tested TGF-β1 using osteoblast-like cells obtained from human fetuses as well as THP-1 cells. TGF-β1 increased IL-1β+DEX-induced aromatase activity in osteoblast-like cells, while it inhibited activity in skin fibroblasts. Similar enhancement of aromatase activity by TGF-β1 was found in DEX-stimulated THP-1 cells and this cell line was used for further experiments. In THP-1 cells, TGF-β1 enhanced DEX-induced aromatase activity almost linearly by 12 h and thereafter. Increased levels of P450arom transcripts were also demonstrated by RT-PCR at 3 h of TGF-β1 treatment and thereafter. Cyclohexamide abolished enhancement of activity but did not inhibit the accumulation of P450arom transcripts induced by TGF-β1. Increase in P450arom expression by TGF-β1 was attributable to expression driven by promoter I.4. TGF-β1 did not change the half life of P450arom transcripts. To identify the cis-acting elements responsible for TGF-β1 action on aromatase expression, transient transfection assays were performed using a series of deletion constructs for promoter I.4 (P450-I.4/Luc). Two constructs (-410/+14 and -340/+14) that contain a functional glucocorticoid response element (GRE) and downstream sequence showed significant increase of luciferase activity in response to TGF-β1. Deletion and mutation of the GRE in P450-I.4/Luc (-340/+14) abolished the TGF-β1. The luciferase activity of a (GRE)1-SV40/Luc construct was also stimulated by TGF-β1. These results indicate that TGF-β1 increases the expression of P450arom at the level of transcription through promoter I.4, at least in part via an enhancement of transactivation activity of the GR in THP-1 cells. TGF-β1 is suggested to be one of the physiological up-regulatory factors of bone aromatase.


http://www.sciencedirect.com/science/article/B6T3G-47NWFBW-36/2/227ad7e2ad7c8488900daff83269b999a

The apparent preferential expression of the elastase/cathepsin G protease inhibitor antileukoproteinase (ALP) in endometrium of species with epitheliochorial placenta suggests mechanisms of transcriptional regulation unique to these mammalian species. To begin to define the cis-acting regulatory elements involved in the endometrial transcription of the ALP gene, the
porcine ALP gene was isolated and characterized. The porcine gene spans at least 13 kb and consists of 5 exons and 4 introns. This genomic structure, except for an additional exon, is similar to that of the human gene where the first three exons encode the signal peptide, trypsin/cathepsin G binding region, and elastase binding region, respectively. The positions of the 16 cysteine residues in exons 2 and 3 of the human gene are conserved in the porcine gene. The porcine gene contains a TATA box at -29 nucleotide (nt), and sequences with limited homology to those which might bind the transcription factors AP-1, AP-2, Sp-1 and Oct-1. The functional promoter activity of the ALP-5' flanking DNA was examined using chimeric ALP-chloramphenicol acetyl transferase (CAT) DNA constructs, after transient transfection in human (ECC-1, Ishikawa) and rabbit (HRE-H9) endometrial and human trophoblastic (JEG-3) cell lines. A 887 nt fragment of the ALP-5'-flanking region (-887ALP-pCAT-E) was active in these cell lines, with the highest promoter activity observed in the ECC-1. Progressive 5' deletion of the 887 nt fragment up to -243 nt had no effect on CAT gene expression in all cell lines, relative to the longest construct. Results suggest that the approximately 240 bp fragment most proximal to the transcription initiation site confers basal and limited endometrial tissue-specific promoter activity to the ALP gene 5'-flanking region. These studies also establish the ECC-1 cell line as an in vitro model system to elucidate the control of ALP gene transcription in the endometrium.


http://www.sciencedirect.com/science/article/B6T3G-3WM5C4D-K2/2/22aada3530f90294fd330c5ca1bd6beca

The identification of estrogen-responsive genes in the heart, is necessary to understand estrogen-induced changes in cardiac function. Using Delta RNA fingerprinting, we demonstrate that a single injection of estradiol benzoate (50 [mu]g, s.c.) revealed mRNA species that were elevated, down-regulated, or were unaffected in the heart tissue of ovariectomized female rats. One of the upregulated genes was identified, by cloning and sequencing, to have 95.8% (230/240) identity with the 3' end of the rat ant1 gene encoding the mitochondrial adenine nucleotide translocator, ANT1. Using the isolated ANT1 cDNA (280 bp) as a probe in Northern analysis, estrogen was shown to upregulate the expression of cardiac ANT1, by at least 3-fold in female rats, from as early as 1 h to as long as 24 h. In contrast, estrogen treatment had no effect on ANT1 expression in heart tissue from male rats. RNA yields were low in rat atria and no transcript was detectable by Northern analysis. Using primers specific to the known rat ANT1 gene, the estrogen upregulation of the cardiac ANT1 transcript in female rat was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR); a predicted product of 249 bp was obtained and this was stimulated by at least 3-fold upon estrogen treatment for 24 h.


The cDNA encoding the precursor polypeptide for schistostatins, allatostatin-like peptides which have been shown to inhibit peristaltic movements of the lateral oviducts of Schistocerca gregaria, has been cloned and sequenced. Translation of this sequence reveals the presence of a pre-proschistostatin consisting of 283 amino acids. It contains ten different peptide sequences which are flanked by dibasic cleavage sites and C-terminal amidation signals. Eight of these peptides were identical to the schistostatins (or Scg-ASTs) that were previously purified from Schistocerca
gregaria brain extracts. Two novel peptide sequences were discovered. One of these is the first AST-like peptide which has a C-terminal valine residue. Two peptides contain within their sequence an internal dibasic site which suggests a possible role for alternative processing and/or degradation. The schistostatin precursor differs from cockroach pre-proallatostatins in size, in sequence and in organization. It contains a lower number of peptides (10 versus 13 or 14) which are interrupted only once by an acidic spacer region (versus four in Diploptera punctata and Periplaneta americana). Northern analysis showed the presence of a 2.4 kb mRNA band in the locust central nervous system and midgut. This indicates that schistostatins, like other ASTs, are a good example of insect brain/gut peptides.


http://www.sciencedirect.com/science/article/B6T3G-3YDGFYR-B/2/330897c3548224195b3e394d5828b522

Rat Zn-15 is a transcription factor activating GH gene expression by synergistic interactions with Pit-1, named for 15 DNA-binding zinc fingers, including fingers IX, X, and XI that are responsible for GH promoter binding. In this study, a mouse cDNA for Zn-15 was characterized. The predicted 2192-amino acid mouse protein is 89% identical to rat (r) Zn-15 overall, and is 97% similar in the C-terminal domain necessary for binding the GH promoter. However, the mouse cDNA encodes 16 zinc fingers, and sequences of rZn-15 pituitary cDNAs were the same as the mouse (m) Zn-16; the rat sequence in GenBank has a one nucleotide offset of a 17-bp segment in the finger V region. The mouse and corrected rat sequences contain four tandemly repeated fingers in the N-terminus, each separated by seven amino acids, typical of zinc finger proteins of the transcription factor IIIA-type. Analysis of mZn-16 expression by RT-PCR showed that the mRNA is produced at similar levels in normal and GH-deficient Ames dwarf (Prop-1) mouse pituitaries at postnatal day 1. Mouse Zn-16 mRNA also was detected by ribonuclease protection assay in the pre-somatotrophic mouse cell line GHFT1-5. The Zn-16 protein is bipartite in that the N-terminal half displays tandem spacing typical of most zinc finger proteins, while the C-terminal portion contains long linkers between fingers that cooperatively bind to a DNA response element. Expression in early postnatal pituitary and in pre-somatotrophic cells suggests that Zn-16 could play a role in pituitary development prior to somatotroph differentiation.

Voss, T. C., L. R. Goldman, et al. (2001). "GH mRNA levels are elevated by forskolin but not GH releasing hormone in GHRH receptor-expressing MtT/S somatotroph cell line." Molecular and Cellular Endocrinology 172(1-2): 125.


The MtT/S somatotroph cell line should be a growth hormone-releasing hormone (GHRH)-responsive model system for the study of physiological control of growth hormone (GH) transcription because GH secretion from these cells is stimulated by GHRH. To examine the GH transcriptional activity of these cells, endogenous GH mRNA levels were measured using a ribonuclease protection assay following treatment under a variety of hormonal conditions. While omission of serum led to reduction of GH mRNA to 22% of control levels by 2 days and to 8% by 5 days (P=20 nmol/106 cells per h in both serum-containing and serum-free media, and that GHRH had no effect on cAMP levels, suggesting constitutive activation. To rule out the possibility of autocrine stimulation by GHRH produced endogenously, GHRH mRNA was not detectable in MtT/S cells using RT-PCR amplification. The stimulatory G-protein [alpha] subunit, mutations of
which are known to activate adenylate cyclase constitutively in acromegaly, was sequenced but found not to differ from normal pituitary in the regions most commonly mutated. Finally, treatment with 10 [mu]M forskolin, to directly activate adenylate cyclase, increased GH mRNA to 140% of controls in TDM, and to 163% in serum-free medium after 2 days, and to 166% in TDM-treated cells and 174% in serum-free culture after 5 days (all Pgsp mutations.


http://www.sciencedirect.com/science/article/B6T3G-427JWGY-1S/2/519d326fb21fe9999bd1c0b76558bf34

Gonadotropin-releasing hormone (GnRH) has been reported to exist in many non-hypothalamic tissues, such as the placenta, gonads, and mammary glands, while there still have been no reports concerning the existence and expression of GnRH in the mammalian digestive system. Immunocytochemistry and in situ hybridization results show that GnRH molecule and GnRH mRNA are both exclusively distributed in exocrine pancreas, and RT-PCR result further proves that GnRH transcription units do exist in the pancreas, which possess the same sequence as the hypothalamus GnRH mRNA. Quantitative analysis indicates that mRNA levels in rat pancreas remain at a low level (less than 10% of that in hypothalamus) without sexual or developmental difference. This is the first report suggesting the existence and gene expression of GnRH in rat pancreas.


http://www.sciencedirect.com/science/article/B6T3G-3VTYSMT-8/2/2f88846b64c32c7b4ea8f1b33f27131

A 72 kilobase pair DNA fragment that contains the mouse phosphoenolpyruvate carboxykinase (PEPCK) gene locus, pck1, was isolated from a genomic bacterial artificial chromosome library. The region from ~-5.5 to +6.6 kilobase pairs relative to the pck1 transcription start site was sequenced and exhibits a high degree of homology to the rat and human genes. Additionally, the chromatin structure of the PEPCK gene in mouse liver resembles that seen in rat. Backcross panel analysis of a microsatellite sequence confirms that the gene is located on chromosome 2. Hypersensitive site analysis was performed on nuclei isolated from the adipocyte cell line 3T3-F442A in the preadipose and adipose states. Several hypersensitive sites are present in the undifferentiated 3T3-F442A cells, before PEPCK mRNA is detected. The same sites are present after differentiation, however, the sensitivity of mHS 3 increases relative to the others. We conclude that the chromatin is open in 3T3-F442A cells and that factors are able to bind in the undifferentiated state but that something else is required for transcription.


http://www.sciencedirect.com/science/article/B6T3G-47N6MJR-JG/2/832005b95edd318dd058231bf964f9f1
A sheep testicular cDNA library constructed in pcDNA1 vector was screened with a probe generated by polymerase chain reaction (PCR) and corresponding to a 1.6 kb fragment of the rat luteinizing hormone receptor cDNA. Several clones hybridizing to the rat probe at low stringency were sequenced to obtain 95% of the putative full-length ovine follicle-stimulating hormone receptor (oFSH-R) cDNA. The missing 5’ region was obtained by PCR amplification of the cDNA library. Sequencing revealed a 2085 nucleotide open reading frame encoding a mature protein of 678 amino acids (74,580 daltons). The oFSH-R is remarkably similar (> 90%) to the human and rat FSH receptors, has a structural motif like the G protein-coupled family of receptors and contains 3 potential sites for N-linked glycosylation. RNA blot analysis revealed two major transcripts of 2.6 kb and 6.7 kb in size and a smaller transcript of about 1 kb in the sheep testis. A 53 residue segment in the extracellular domain unique to the receptor contains more than 50% of residues bearing functional side chains that could participate in ligand (FSH) interaction and/or signal transduction. Transfection of human fetal kidney cell line (293) with the cloned oFSH receptor cDNA based in pcDNA1/Neo vector revealed functional expression. Labeled oFSH bound to receptor expressed on the membrane with high affinity and specificity. In stably transfected 293 cells, purified oFSH and hFSH but not oLH stimulated cyclic AMP accumulation. Chemically deglycosylated oFSH (DG-oFSH) was inactive in these cells but it effectively blocked the action of native hormone. Thus, the functional characteristics of the cloned receptor are similar to the natural receptor in testis.