CONFERENCE

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This was the 13th meeting of this biennial series dedicated to the biochemistry and molecular biology of steroid hormones and was held in Monte Carlo. The impressive recent progress in the field was well summarized by 35 symposium speakers and 95 poster presentations. Twelve symposium lectures were devoted to steroid receptor structure and gene regulation, thus illustrating the particularly rapid development in this area of steroid research.

Major progress has been made in the identification and elucidation of the mechanisms of action of coactivators and corepressors of steroid receptor action. In fact, as indicated by B. O'Malley (Houston, TX, USA), the 'majority of the amplification of response to progesterone receptor action comes from coactivators. The addition of steroid receptor coactivator 1 (SRC1) and CREB-binding protein (CBP) can synergize to give up to a 40-fold increase in progesterone-induced induction of transcription over that observed with the progesterone receptor alone'. Fig. 1 is a diagrammatic representation of today's knowledge and a look into the near future about the coregulation of steroid receptor-mediated gene transcriptional activity. R. Losson (Strasbourg, France) described the topography of the multiple protein–protein interaction sites of TIFloc, a chromatin-specific mediator of the ligand-dependent activation function AF-2 of steroid and non-steroid receptors.

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D. McDonnell (Durham, NC, USA) emphasized the importance of receptor-associated proteins in estrogen receptor (ER) action. He also described human repetitive ALU sequences containing ER elements, which could account for various degrees of sensitivity to estrogens in different individuals. The complement 3 gene is expressed in bone and is estrogen-sensitive, thus providing an assay for the assessment of agonist/antagonist activities of various antiestrogens. The AF-1 activity of the ER is not required for the bone-sparing activity of raloxifene. Human macrophage inflammatory protein (HMIP) expression is stimulated by ICI 182780 as well as by estradiol, thus illustrating the complexity of ER action. Different antiestrogens drive the receptor into specific conformations that lead to various degrees of inactivation or activation of each target gene (in each cell type of each species). Acetylation of histones induced by SRC-1 (and a series of other factors) loosens the nucleosome, thus dramatically facilitating the action of polymerase to read and transcribe the gene, rapidly and repeatedly. Conversely, the interaction of the antiprogestin RU486 with the progesterone receptor (PR) leads to the opposite reaction, namely histone deacetylation and repression of gene activation.

G. Hager (Bethesda, MD, USA) discussed the interaction of glucocorticoid and progesterone receptors with ordered chromatin templates, using the mouse mammary tumor virus (MMTV) promoter as a model. Receptors induce transcription by two separate pathways. The first event involves a local nucleoprotein remodeling that results in the loss of chromatin repression and loading of secondary transcription factors. This remodeling event is not well understood, but probably involves the reorganization of a higher order chromatin structure rather than simple nucleosome loss. The second stage of activation results from direct protein–protein interactions between receptors, coactivators and the basal transcription complex.

H. Reichardt (Heidelberg, Germany) has generated mice carrying mutations in the dimerization domain of the glucocorticoid receptor (GR) to analyze the role of the GR in various physiological functions. This mutation is not lethal but still leads to important deficiencies, especially in the pituitary–adrenocortical system.

D. DeFranco (Pittsburgh, PA, USA) described the identification of a point mutation within the DNA-binding domain of the rat GR (arginine at position 496; R496) that exerted dramatic effects on subnuclear targeting of the receptor. Carboxyl-terminal truncated rGRs possessing various point mutations at R496S localized within a limited number of large foci within nuclei of transiently transfected Cos-1 cells. The 70-kDa heat shock protein, hsp70, also colocalized within R496 mutant foci. The mistargeting of R496 mutant receptors was relieved upon overexpression of a DNAJ homolog (HDJ-2/HSDJ). Overexpression of HDJ-2/HSDJ also corrected defective transactivation and transrepression activity of R496 mutant GRs.

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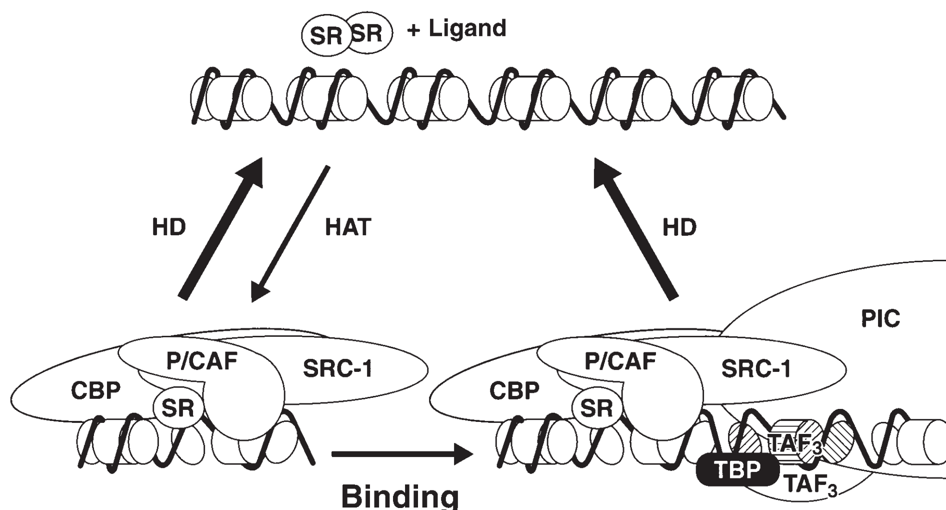

Figure 1. A two-step model for activation of transcription. Abbreviations: CBP, CREB-binding protein; HAT, histone acetyltransferase; HD, histone deacetylase; P/CAF, p300/CBP-associated factor; SR, steroid receptor; SRC-1, steroid receptor coactivator-1; TAF_3, TBP-associated factor; TBP, TATA-binding protein.

J. Baxter (San Francisco, CA, USA) presented results of crystallographic studies of the thyroid hormone receptor ligand and DNA-binding domains, and a detailed mutational analysis of the receptor surface. These results have provided new information about ligand binding, and a better definition of the receptor domains required for the hinge function, homo- and heterodimerization and the AF-2 transcription function.

Although mutations of the androgen receptor (AR) appear to be involved in only a small proportion of prostate cancers, such mutations provide important information about the role of various regions of the AR in the specific actions of the molecule. A. Cato (Karlsruhe, Germany) has found that 5α-dihydrotestosterone (DHT) can initiate a cascade leading to the activation of mitogen-stimulated (MAP) kinase in human prostate cancer cell lines. The activation of MAP kinase by DHT was not inhibited by antiandrogens, such as hydroxyflutamide and cyproterone acetate, and was even augmented by the antiandrogen Casodex. This offers a possible explanation for the resistance to antiandrogen therapy after long-term treatment of prostate cancer.

D. Edwards (Denver, CO, USA) has shown that chromatin high mobility group (HMG) proteins 1 and 2 facilitate binding to DNA of the ER, AR, PR and GR. HMG 1/2 increased PR transactivation by a factor of nine compared with progesterone alone. HMG 1/2 thus appear to serve as common coactivators of the steroid subgroup of the nuclear receptor superfamily.

J. Kato (Chiba and Yamanashi, Japan) described the structure of various ER mRNAs, specifically the multiple untranslated exons and promoters that result from alternative splicing. Such mechanisms appear to regulate the region- and tissue-specific expression of the ER gene in the brain and peripheral tissues in the rat and monkey.

E. Castano (Rochester, MN, USA) and A. Munck (Lebanon, NH, USA) provided new information on the role of phosphorylation in steroid receptor action. In particular, Dr Castano noted that phosphorylation at specific sites of the ER modulates the activity of the receptor while providing an opportunity for the MAP kinase pathway to influence the ER and other steroid receptor pathways. Dr Munck described how phosphorylation of the glucocorticoid receptor alters its activity and stability, and varies throughout the cell cycle.

J. Drouin (Montreal, QC, Canada) described a novel dimeric mechanism of action of the orphan receptor Nur77 (also known as NGFLB). This mode of DNA binding and transcriptional activation is much more potent than that of Nur77 action as a monomer. GR and Nur77 compete for each other's action, and thus serve to mediate the effects of corticotropin-releasing factor (CRF) and glucocorticoids in the control of adrenocorticotropin (ACTH) secretion by the pituitary gland.

As discussed by B. Desvergne (Lausanne, Switzerland), three subtypes of peroxisome proliferator-activated receptor (PPARα, -β and -γ) have been characterized. These form heterodimers with the RXR and bind to a DNA sequence formed from two hexanucleotides in a direct repeat orientation where PPAR occupies the 5′ half-site and RXR the 3′ half-site. The activation by fatty acids or fibrates of PPARα triggers an increase in fatty acid oxidation pathways. In contrast, PPARγ is involved in adipose tissue differentiation and lipid storage. The design of a new *in vitro* ligand-binding assay, based upon the ligand-dependent interaction of PPAR with the coactivator SRC-1, provides a tool for screening new drugs that potentially act on lipid metabolism.

D.W. Hum (Ste Foy, QC, Canada), in collaboration with Alain Belanger's group, described a new uridine diphosphate (UDP)-glucuronosyltransferase specific for androgens, especially androsterone (UGT2B17). The expression of this isoenzyme is thought to play an important role in the termination of the androgen signal, and its expression is suppressed by androgens. Transfection studies have shown that UGT2B17 inhibits the androgenic response in human prostate cancer cells. This is an example of intracrinology (Fig. 2), whereby active steroids are made locally in peripheral tissues from the inactive adrenal steroid precursors, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEA-S). These locally made steroids activate their specific receptors in the same cells in which they are synthesized. Subsequently, the active androgens are glucuronidated by uridine glucuronosyl transferase (UGT) enzymes, such as UGT2B17, which catabolizes the

active steroids intracellularly before their diffusion out of the cells and their excretion by the liver and kidney.

As another example of intracrinology, A. Naray-Fejes-Toth (Lebanon, NH, USA) has shown that the transformation of cortisol to cortisone by 11β-hydroxysteroid dehydrogenase (11β-HSD) leaves the mineralocorticoid receptor free of cortisol and able to interact solely with aldosterone. Otherwise, mineralocorticoids cannot exert their modulatory role because of the potential saturation of the mineralocorticoid receptor by large amounts of cortisol. Type 2 11β-HSD is the isoenzyme responsible for this intracrine mechanism, and it is expressed in the cortical collecting ducts of the kidney. It is localized exclusively to the endoplasmic reticulum and the plasma membrane in both kidney epithelial and transfected cells, while the cell nucleus and other organelles are negative.

The hypothalamic control of steroid secretion was addressed by studies using hypothalamic neurons (GT1 cells) that secrete gonadotropin-releasing hormone (GnRH), the neurohormone responsible for stimulating the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. GT1 cells are a useful model, as they release GnRH in a pulsatile fashion under *in vitro* incubation conditions, in a manner similar to that observed *in vivo*. K.J. Catt (Bethesda, MD, USA) reported that the autocrine actions of GnRH agonists on GT1-7 cells, which express GnRH receptors and respond to agonist stimulation with changes in the secretory profile of GnRH, are also seen in cultured hypothalamic neurons from fetal rats. The expression of GnRH receptors in hypothalamic neurons was demonstrated by the polymerase chain reaction (PCR), as well as by radioligand binding studies. Treatment of perifused hypothalamic neurons with GnRH antagonists abolished pulsatile secretion of GnRH from both GT1 cells and other hypothalamic neurons, indicating the role of autocrine feedback by endogenous GnRH in the generation of pulsatile neurosecretion.

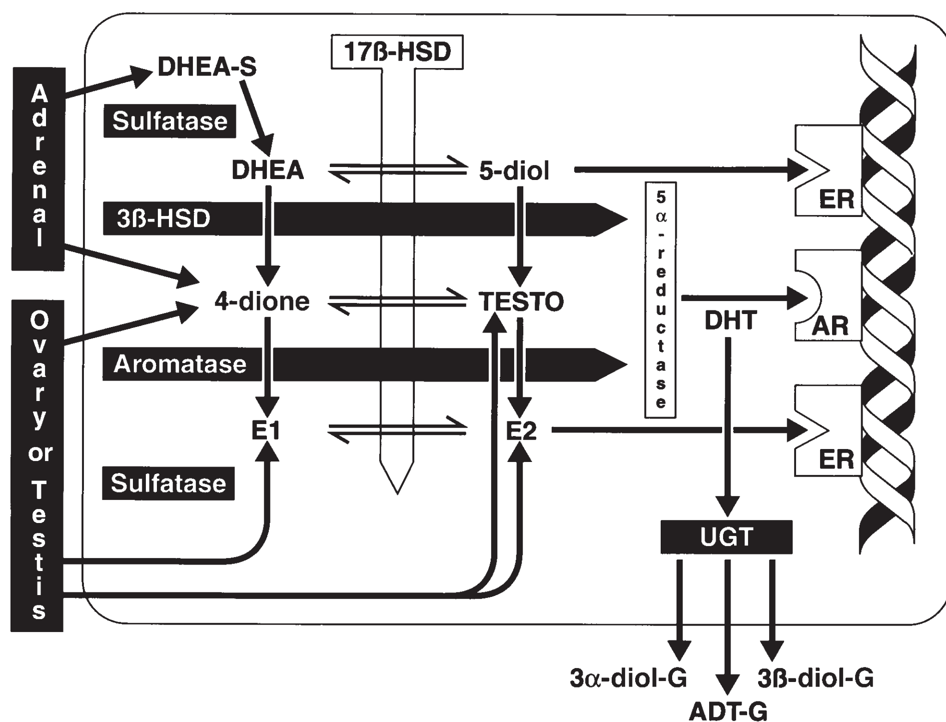

Figure 2. Intracrine metabolism of steroids in target tissues. Abbreviations: ADT-G, androsterone glucuronide; AR, androgen receptor; DHEA, dehydroepiandrosterone; DHEA-S, DHEA-sulfate; DHT, dihydrotestosterone; ER, estrogen receptor; E1, estrone; E2, 17β-estradiol; TESTO, testosterone; UGT, steroid UDP glucuronosyltransferase; 3α-diol-G, 5α-androstane-3α-17β-diol-glucuronide; 3β-diol-G, 5α-androstane-3β-17β-diol-glucuronide; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 4-dione, 4-androstenedione; 5-diol, 5-androstene 3β-17β-diol; 17β-HSD, 17β-hydroxysteroid dehydrogenase.

L. Martini (Milan, Italy) described the brain areas that contain aromatase activity, especially the hippocampus. Gonadotropin secretion and sexual behavior are modulated by aromatase activity and estrogen formation in the brain. The enzyme 5α-reductase is present mainly in neurons, while 3α-11-HSD is present mainly in astrocytes. Progesterone appears to be the main substrate of 5α-reductase in the brain. GT1 cells (GnRH-secreting neurons) possess 5α-reductase but not aromatase activity. L. Jung-Testas (Paris, France) presented data showing the formation of steroids in primary cultures of rat glial cells, and the presence of steroid hormone receptors. As an example, progesterone and insulin synergize to stimulate the expression of myelin proteins such as myelin basic protein (MBP) and cyclic nucleotide phosphodiesterase (CNP-ase).

M. Kawata (Kyoto, Japan) described the localization of the glucocorticoid receptor protein and mRNA in different parts of the brain, the effect of glucocorticoids on the hippocampus and the effect of estrogens on the morpho-functional aspects of the hypothalamus. A.F. De Nicola (Buenos Aires, Argentina) described tropic effects of glucocorticoids in the central nervous system, mediated by increased expression of the sodium pump and neurotropins. Moreover, a sex-dependent regulation by glucocorticoids of vasopressin mRNA expression was also postulated.

G. Emons (Marburg, Germany) described the presence of high-affinity GnRH receptors in breast, ovarian, endometrial and prostate cancer cell lines. Moreover, the normal endometrium and the ovary possess GnRH receptors and secrete GnRH, thus suggesting a physiological role for the neuropeptide in these tissues. Both GnRH agonists and antagonists inhibit the proliferation of breast, ovarian and endometrial cancer cell lines. The mechanisms involved appear to be mediated by growth factor signaling pathways. Such effects might have an

important role in the treatment of cancer of these tissues with GnRH agonists.

J-P. Raynaud (Paris, France) presented data obtained with a new transdermal norethisterone acetate (NETA) formulation and the possible use of a weekly application of the progestin patch. In 395 postmenopausal women receiving Estraderm and randomized to oral Promegestone or the transdermal NETA patch, clinical tolerance, safety and efficacy were similar between groups.

R. Delansorne (Monaco), on behalf of J.K. Williams (Winston-Salem, NC, USA), presented data on the protective action of estrogens on atherosclerosis and paradoxical coronary reactivity to acetylcholine in the ovariectomized *Cynomolgus* monkey. Progesterone did not interfere with estrogens on these endpoints, whereas medroxyprogesterone acetate partially reversed their beneficial effects. However, like progesterone, another orally active synthetic progestin, nomegestrol acetate, did not show any harmful effects on estradiol-induced protection against paradoxical coronary reactivity. Isoflavonoids found in soybean protein extracts showed beneficial effects on plasma lipoproteins as well as on vascular reactivity. Improved vascular reactivity was also found with pure genistein.

J. Simard (Ste Foye, QC, Canada) described the stimulatory effects of interleukin 4 (IL-4) and IL-6 on the transformation of the potent estrogen 17β-estradiol into the weak estrogen estrone, a reaction that is catalyzed by type 2 17β-HSD. In addition, these two interleukins inhibit the proliferation of a breast cancer cell line (ZR-75-1). On the other hand, IL-4 and IL-13, but not IL-6, induce the expression of the 3β-HSD gene in human breast cancer cell lines (ZR-75-1, T-47D and MDA-MB-231) as well as in normal human mammary epithelial cells in primary culture. Evidence was presented for stimulation of Stat-6 binding to DNA following treatment of ZR-75-1 cells with IL-4 and IL-13. Induction of 3β-HSD gene expression by IL-4 was also observed in a large series of cancer cell lines.

R.L. Sutherland (Sydney, Australia) described how the mitogenic effects of estrogens affect kinases that induce the phosphorylation of the retinoblastoma gene product (RB). Activation of both cyclin Dl–CDK4 (cyclin-dependent kinase 4) and cyclin E–CDK2 occurs early in the estrogenic response. The former kinase appears to be activated by increased cyclin Dl gene expression, while cyclin E–CDK2 activation involves the formation of high specific activity complexes devoid of the CDK inhibitors p2l and p27.

In an overview of recent results from their laboratory, H. Rochefort and collaborators (Montpellier, France) addressed the question of the role of estrogens in cell invasion through the basement membrane, and in cell motility. Using the Boyden chamber assay, they consistently observed estradiol-induced inhibition of cancer cell invasion and motility. At present, the mechanism of this inhibitory effect of estrogen is not known. They concluded that estrogens may have opposite effects on ER-positive breast and ovarian cancers, as they facilitate tumor growth but inhibit invasion and motility. This is consistent with the good initial prognosis of ER-positive breast cancers noted in several clinical studies, and with the loss of heterozygosity found in 6q24 in aggressive breast and ovarian cancers.

D. Berliner (Menlo Park, CA, USA) described the functionality of the human vomeronasal organ (VNO) when exposed to a variety of vomeropherins (either pheromones or synthetic analogs). This work demonstrated the following: (1) the human VNO is functional in adult humans and is markedly gender specific; (2) the putative human vomeronasal receptors are highly specific; (3) the VNO can modulate autonomic reflexes and induce behavioral responses; (4) patients with hypogonadotropic hypogonadism do not have a functional vomeronasal system; and (5) hypothalamic–pituitary–gonadal function can be modulated through the VNO. The authors conclude that the human VNO is a functional chemosensory organ that modulates hypothalamic function through afferent nerve impulses.

L. Murphy (Winnipeg, Man, Canada) described the pathophysiological role of ER variants in human breast cancer. One mechanism underlying altered ER signal transduction might be altered expression of ER variants. Several types of altered ER mRNA species have been detected in both normal and neoplastic breast tissues. ER variant mRNAs are also expressed in normal human breast tissue, and the levels of some (clone 4 truncated and exon 5 deleted ER mRNAs) but not all (exon 7 deleted ER mRNA) variant mRNAs are significantly elevated in human breast tumors. More recently, an association of overexpression of an ER variant mRNA (an exon 3 + 4 deleted ER) with the development of estrogen-independent growth in a human breast cancer cell line model was found. Such data are consistent with a role(s) for ER variants in human breast tumorigenesis and breast cancer progression.

J. Swinnen (Leuven, Belgium) has demonstrated that androgens induced a marked accumulation of neutral lipids in human prostate cancer cells. His work has provided evidence for the existence of a novel cascade mechanism by which androgens, mediated by the recently characterized sterol regulatory element-binding proteins, coordinately stimulate the expression of enzymes belonging to the two major lipogenic pathways: fatty acid synthesis and cholesterol synthesis.

D. Tonetti (Chicago, IL, USA) compared the actions of tamoxifen and toremifene on the EnCa 101 human endometrial tumor model and found that both antiestrogens have similar growth-stimulatory effects. The potential mechanism of antiestrogen-stimulated endometrial tumor growth was investigated by examining the role of known activators of the AP-1 signal transduction pathway, the protein kinase C (PKC) family of isoenzymes, in the EnCa 101 human endometrial tumor model. This work indicated that increased PKC isoenzyme expression correlates with hormone-independent breast cancer, as well as antiestrogen-stimulated endometrial cancer growth.

J. Pasqualini (Paris, France) discussed the remarkable development in this synthesis of new progestins over recent years. With regard to breast cancer, information on the action of progestins is limited to the positive response observed with the progestins, medroxyprogesterone acetate and megestrol acetate, in postmenopausal patients with advanced breast cancer. In hormone-dependent breast cancer cells, various progestins (nomegestrol acetate, tibolone and promegestone) are potent sulfatase inhibitory agents. It was also demonstrated that these progestins are active in inhibiting the conversion of estrone to estradiol by 17β-hydroxysteroid dehydrogenase. Consequently, blockade of the formation of estradiol via sulfatase by progestins might provide interesting, new possibilities for clinical applications in breast cancer.

J. Cidlowski (Research Triangle Park, NC, USA) emphasized the importance of apoptosis in the development and homeostasis of total organism cell number, as well as in the editing of damaged or mutant cells. Current studies are aimed at identifying the molecular entities that carry out apoptotic processes, and the understanding of their activation by various apoptotic signals. T lymphocytes serve as an excellent model for such studies because they undergo apoptosis in response to glucocorticoids and other compounds. In T lymphocytes, DNA fragmentation and caspase activation occur only in cells that have undergone a reduction in cell volume. In addition, recent evidence has indicated that early ion fluxes are critical for the activation of apoptotic pathways. L.B. Hendry (Augusta, GA, USA) reported that a key step in gene regulation by steroids is the binding of hormone to receptors that interact with DNA. Steroids insert stereospecifically between base pairs, and this fit correlates with activity. It was proposed that receptors mediate insertion of hormone into DNA, and that this step is rate limiting.

A.M. Soto (Boston, MA, USA) described the manner in which the endocrine and reproductive effects of environmental agents such as pesticides and industrial chemicals are believed to affect wildlife and humans. Such compounds have the ability to: (1) mimic the effects of endogenous hormones; (2) antagonize the effects of endogenous hormones: (3) disrupt the synthesis and metabolism of endogenous hormones; and (4) disrupt the synthesis and metabolism of hormone receptors. A single chemical can produce neurotoxic, estrogenic and antiandrogenic effects. The systematic identification of these compounds before they are introduced into widespread use will require the collaboration of the scientific community, government agencies, the industrial sector and the public at large.

The 1998 Neuroendocrine Workshop on Stress: a Report on the American Neuroendocrine Society Annual Workshop

James I. Koenig

Since Hans Selye first defined the stress response[1], investigators have pursued the identification of the major biochemical mediators and the loci of their effects within the brain tirelessly. In recent years, these efforts have culminated in the identification of corticotropin-releasing hormone (CRH) and vasopressin (AVP) as the main mediators of the response to stress. A newly added mediator whose physiological function in the stress response is yet to be established is urocortin[2]. CRH and urocortin exert their effects by binding to four different CRH receptors in brain and pituitary, as well as binding to CRH-binding protein, which modifies the actions of CRH.

The 1998 Neuroendocrine Workshop on Stress was held in New Orleans, LA, USA, from 21–23 June 1998, and was attended by over 125 investigators. The workshop focused on the latest developments in the neuroendocrinology of stress and on the identification, function and clinical utility of these moieties for central nervous system (CNS) and peripheral disorders.

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- **Molecular Mechanisms of Central Stress Integration**

The 41-amino acid peptide CRH, isolated and characterized originally by Vale *et al.*[3], is the most prominent stimulatory factor controlling pituitary adrenocorticotropin (ACTH) secretion. Previous studies have documented clearly that expression of the CRH gene and the subsequent secretion of CRH by hypothalamic neurons are both under the strong negative influence of the adrenal glucocorticoid hormones, cortisol and corticosterone. However, in non-hypothalamic regions of the brain, such as the amygdala, glucocorticoids exert a stimulatory effect on CRH gene expression. This tissue-specific regulation could be a direct effect of the activated glucocorticoid receptor on the CRH gene, or as discussed by Dr A. Seasholtz (Ann Arbor, MI, USA), both the positive and negative effects of glucocorticoids might arise as a result of novel interactions between the glucocorticoid receptor and cAMP-dependent mechanisms, most likely involving novel interactions with cAMP response

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