

## Ancient Glucocorticoid Regulation of CRF?

Glucocorticoids modulate the activity of the hypothalamo-pituitary-adrenal axis, limiting the magnitude and duration of the endocrine stress response. In mammals, these hormones regulate hypophysiotropic corticotropin-releasing factor (CRF) neurons in the paraventricular nucleus of the hypothalamus via negative feedback. By contrast, glucocorticoids positively regulate CRF expression in neurons of the amygdala and bed nucleus of the stria terminalis—brain regions involved in stress-related behaviors such as fear and anxiety.

Because very little is known about how glucocorticoids regulate CRF in non-mammalian vertebrates,

Robert J. Denver, Ph.D., of the University of Michigan in Ann Arbor, and his associates, studied glucocorticoid action on CRF expression in several brain regions of the frog *Xenopus laevis*, using analyses of CRF primary transcript, messenger RNA, and protein, and determined other glucocorticoid actions. Treatment with corticosterone decreased CRF expression in the anterior preoptic area—a region of the frog brain homologous to the mammalian paraventricular nucleus, whereas the corticosteroid synthesis inhibitor metyrapone increased it. Corticosterone also reduced CRF core promoter activity in

transfected tadpole brain in vivo. By contrast, corticosterone increased CRF immunoreactivity in the amygdala and bed nucleus of the stria terminalis, and metyrapone decreased it.

In an article to be published in *Endocrinology*,\* the researchers report that their work provides several lines of evidence suggesting evolutionary conservation of the function of the limbic system. CRF is expressed in the amygdala and bed nucleus of the stria terminalis of the frog, and its expression is increased following exposure to glucocorticoids.

The findings argue against the view that large changes occurred in the

functional organization of the amygdala in the amphibian-reptilian transition. They suggest that the neural cell-type specificity and molecular mechanisms of glucocorticoid-dependent CRF regulation are phylogenetically ancient, and that the limbic pathways mediating behavioral and physiological responses to stressors were likely present in the earliest land-dwelling vertebrates. ■

\* Yao M, Schulkin J, Denver RJ. Evolutionarily conserved glucocorticoid regulation of corticotropin-releasing factor expression. *Endocrinology*, in press.

## Nonhormonal Activity of Male Contraceptive

In the continuing hunt for male oral contraceptives, researchers are working on the *l*-isomer of the indeno-pyridine CDB-4022. Studies in rats and monkeys have shown that *l*-CDB-4022 does not alter endogenous testosterone levels, instead acting on Sertoli and germ cells, which compose the seminiferous epithelium of the testis. Adherens junctions (AJs) connect Sertoli and germ cells and play a major role in spermatogenesis. They include the protein complexes cadherin/catenin, nectin/afadin, and integrin/laminin. The testis also has dynamic pro-survival and pro-apoptotic systems, which balance each

other to regulate germ cell apoptosis. Working with rats, Sailaja Koduri, Ph.D., at BIOQUAL, Inc., in Rockville, Md., and her colleagues studied the effect of a single oral dose of *l*-CDB-4022 to determine the mechanism of its anti-spermatogenic activity. The results of their study will appear soon in *Endocrinology*.\*

The researchers found that *l*-CDB-4022 did not change body weight gain, but did cause loss of testes weight and altered selected serum hormone levels: inhibin B decreased and follicle-stimulating hormone increased, but activin A, testosterone, and luteinizing hormone levels were unchanged relative to controls.

The team detected

ERK1/2 phosphorylation in testes lysates of *l*-CDB-4022-treated rats, beginning 4 hours after dosing. *l*-CDB-4022 altered AJ protein levels, decreasing amounts of the nectin/afadin complex, and increasing integrin- $\beta$ 1, N-cadherin,  $\alpha$ -catenin, and  $\beta$ -catenin levels. Fas ligand and receptor expression rose, and the ratio of membrane to soluble stem cell factor (SCF) mRNA diminished in testes lysates of *l*-CDB-4022-treated rats. Immunohistochemical analysis showed that *l*-CDB-4022 dramatically disrupted the Sertoli cell microtubule network.

The researchers conclude that *l*-CDB-4022 causes germ cell loss from the seminiferous epithelium

by activating the MAPK pathway, reducing the expression of pro-survival factors such as the membrane form of SCF and inducing the pro-apoptotic factor Fas, altering expression of Sertoli-germ cell AJ proteins, and disrupting Sertoli cell microtubule structure. ■

\* Koduri S, Hild SA, Pessaint L, Reel JR, Attardi BJ. Mechanism of action of *l*-CDB-4022, a potential nonhormonal male contraceptive, in the seminiferous epithelium of the rat testis. *Endocrinology*, in press.

