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Interactions among steroid and peptide hormones in osteoporosis and breast cancer

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Creatine kinase B as a marker of cell stimulation

The brain type isozyme of creatine kinase (CKB), a cytosolic enzyme which catalyzes the reversible transfer of a high energy phosphate group between phosphocreatine and ADP, is involved in the regeneration of ATP and the buffering and transport of cellular energy. Its activity is rapidly stimulated by the widest variety of regulators, including steroid and peptide hormones and growth factors; its stimulation parallels in many cases the mitotic stimulation measured by increased DNA synthesis. We are investigating the transcriptional control of the CKB gene, using estrogen as our primary stimulant. We have identified multiple, transferable negative regulatory sequences (silencers) in the 5' flanking region of the CKB gene, upstream of the estrogen response element, capable of being neutralized by excess of the transcriptional coactivator, TIF-2.

Parathyroid hormone fragments as anabolic agents

Measuring stimulation of CKB activity and DNA synthesis, we showed previously that parathyroid hormone (PTH) acts as a mitogen for osteoblasts via a phospholipase C pathway, and not via cyclic AMP, which triggers bone resorption by osteoclasts. In collaboration with colleagues at the Gesellschaft fur Biotechnologische Forschung, Braunchsweig, Germany, we have shown that midregion fragments of human PTH, which lack the N-terminal amino acids necessary for the stimulation of adenylyl cyclase, act exclusively as mitogens. Substitution of amino acids in human PTH (28-48) which resulted in increased resistance to proteolysis produced variants that stimulated skeletal systems at two orders of magnitude lower concentration than the wild type fragment. Midregion fragments given daily to prepubertal rats, for two weeks, increased the thickness of the cortical bone in the humerus and the number of cells in the proliferating zone of the humeral growth plate.

Synergy between vitamin D and estrogens

Our finding that pretreatment with vitamin D increases the anabolic responsiveness of skeletal derived cells to estrogen led to testing analogs of vitamin D which do not share its property of causing excess calcium deposits. Reputedly non-hypercalcemic analogs of vitamin D were shown to increase responsiveness to estrogen and SERMS in cell culture and in immature female rats. However, these analogs showed toxicity after repeated injection. In collaboration with Prof. Gary Posner of Johns Hopkins University, we have found that demonstrably non-calcemic new hybrid analogs of 1,25 dihydroxy vitamin D can increase the responsiveness to estradiol permitting the use of lower concentrations of estrogen against osteoporosis.

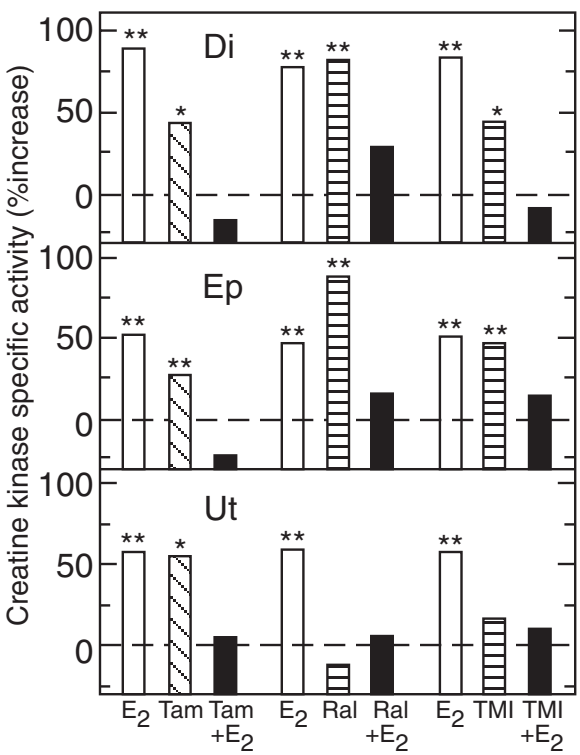

Fig. 1 *Mutual annihilation of stimulation of CK specific activity in diaphysis (Di); epiphysis (Ep) and uterus (Ut) of prepubertal rats. Rats were killed 24 h after i.p. injection of 10 nmol 17 β-estradiol. E2, 1μmol tamoxifen (Tam), raloxifene (Ral), tamoxifen methiodide (TMI) or a combination of E2 and a SERM. *, P * 0.05; **, P * 0.01 vs. vehicle control groups.*

Paradoxical interactions between estrogens and antiestrogens

We have described the phenomenon of mutual annihilation of action between 17 β estradiol and a selective estrogen receptor modulator (SERM) in prepubertal rat diaphysis, epiphysis and uterus and in ROS 17/2.8 cells. CKB activity was stimulated by raloxifene, tamoxifen and tamoxifen methiodide to a specific activity equal to or greater than that induced by 10 nM 17 β estradiol. However, when a fully inhibitory dose of any of these SERMS was given simultaneously with 17 β estradiol, no stimulation of CK activity resulted. Therefore, SERMS were agonists when acting alone, but antagonists to high doses of estrogen. It is expected that excess tamoxifen would prevent the action of a SERM, but that the agonist activity of a SERM is abolished by one thousand-fold less estrogen is as yet unexplained. When ckb-CAT reporter plasmid plus a human estrogen receptor expression plasmid were transfected transiently into MCF-7 cells, a 1:10 ratio of 17 β estradiol to tamoxifen produced mutual annihilation, but the same ratio in ROS 17/2.8 or HeLa cells led to synergistic stimulation. We speculate that, in the presence of estradiol and a SERM, not only active homodimers would be formed, but also hetero-dimers of estrogen-liganded and tamoxifen-liganded receptor monomers. The resulting hetero-dimer confirmation would change the specific receptor surface for interactions with co-activators and co-repressors, structural changes which could help to explain the mutual annihilation and synergy phenomena and their cell selectivity.

Selected Publications

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