FASER MEETING

MOLECULAR CARDIOLOGY: THE BEAT GOES ON

WASHINGTON, D.C.—Recombinant DNA techniques have given cardiac physiologists their first access to the genes, proteins, and chemical signals that regulate the human heart. Speaking at this year's Federation of American Societies for Experimental Biology Meeting, John Baxter (University of California, S.F., Medical School) and Robert Lefkowitz (Duke University, Durham, NC) demonstrated the potential of this entree to inspire new drug design, and the potentially even more powerful insights it provides into basic physiological molecular-motifs.

Baxter, who is also the chief scientific consultant to California Biotechnology Inc. (Mountain View, CA), along with his academic and industrial colleagues, has been investigating the molecular biology of the angiotensinogen converting enzyme, renin, and of atrial natriuretic factor (ANF), an important cardiac-peptide hormone with a wide range of physiological activities. Renin initiates a regulatory cascade that eventually produces angiotensin II, a potent hypertensive agent. By studying renin's genetic organization, and protein chemistry, Baxter and his colleagues hope to be able to design a rational therapeutic to specifically inhibit this aspartyl protease. Renin is primarily synthesized in kidney cells, from which it is released into the circulation. Although most abundant in kidney, the protein has been found in other tissues. To determine if this renin is endogenously produced or if it makes its way to these cells through the circulation, Baxter used a combination of fluorescent-labeled antibodies and radio-labeled nucleic acid probes derived from renin cDNA. He found both renin message and protein in adrenal glomerulosa, testicular lining cells, and cells of the pituitary and ovary.

At least part of this restricted tissue-specific expression is due to recognition of promoter elements upstream of the renin coding sequence. When human placental cells derived from the amnion or chorion are maintained in culture, chorionic but not amnionic cells synthesize easily detectable levels of renin precursor. When Baxter transfected these cells with plasmids containing the promoter region of the renin gene fused to a chloramphenicol acyltransferase (*cat*) reporter gene, he found that the promoter functioned only in the cell type normally able to synthesize renin. That is, *cat* expression was high in

chorion cells and undetectable in cells of the amnion. Deletion of the promoter resulted in loss of *cat* expression. In related experiments, Baxter has begun to define additional, enhancer-like control sequences, including a glucocorticoid regulatory element, in the renin 5'-flanking DNA.

ANF, an atrial peptide-inhibitor of angiotensin II and a powerful vasodilator, represents the other route to a potential hypotensive these scientists have been studying at the molecular level. It is synthesized as a 126-amino acid protein, which is processed (probably simultaneous with its secretion) into a 28-amino acid active peptide. Like renin, it has a limited but not unique tissue distribution. While cells of the atrium are the primary source of this peptide, small quantities of ANF are synthesized in the ventricle, aortic arch, pituitary, and lung. Since it is unlikely that these levels contribute to circulating ANF, the peptide in these tissues most probably participates in paracrine or autocrine effects. Baxter speculated that, since a common physiological theme to this distribution is pressure sensitivity, ANF may modulate pressure-sensitive phenomena. Indeed, ANF gene expression is positively regulated by increased arterial pressure.

Using bovine smooth muscle cells, John Lewicki at California Biotechnology has recently isolated a gene for a putative ANF-receptor by purifying a high-affinity ANF binding protein and screening a cDNA library with probes designed from its sequence. The best evidence that this cDNA encodes an ANF receptor comes from experiments in which ANF mRNA, transcribed *in vitro*, was microinjected into frog oocytes. After treatment, the oocytes displayed ANF binding activity indistinguishable

from that of smooth muscle cells.

Robert Lefkowitz has been analyzing another receptor system crucial to cardiac physiology, the adrenergic receptors, whose agonists are among the most potent stimulators of renin secretion. Over the past few years, he has been able to purify to homogeneity all three classes of catecholamine receptors—beta, alpha-1, and alpha-2—by developing highly specific affinity chromatographic techniques. The most intensively studied member of this family thus far has been the beta-2 agonist binding activity. This is primarily due, Lefkowitz said, to the successful cloning of the hamster gene about one year ago.

The beta-2 receptor is the only G protein-coupled receptor, other than rhodopsin, whose primary structure is known. And as deduced from the cDNA clone, the two proteins have striking structural similarities: Near the amino terminus are two consensus sites for N-linked glycosylation, followed by a long hydrophobic region with seven potential membrane-spanning domains, and a carboxy-terminus rich in threonine and serine. Additionally, the proteins have considerable sequence homology in the presumptive membrane-spanning loops.

Perhaps the most dramatic evidence that the two proteins share fundamental structure: function relationships involves the agonist-dependent kinases that are responsible for receptor desensitization. Lefkowitz showed that the beta-2 enzyme can phosphorylate the rhodopsin receptor, but only in the presence of light. Conversely, the rhodopsin kinase recognizes the adrenergic receptor as a substrate only when it has bound an appropriate catecholamine.

—Harvey Bialy

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The biosynthesis (*Left*) and multiple activities (*Right*) of atrial natriuretic factor. Circulating ANF increases the glomerular filtration rate, inhibits the action of angiotensin II, and possesses an intrinsic vasodilation activity.