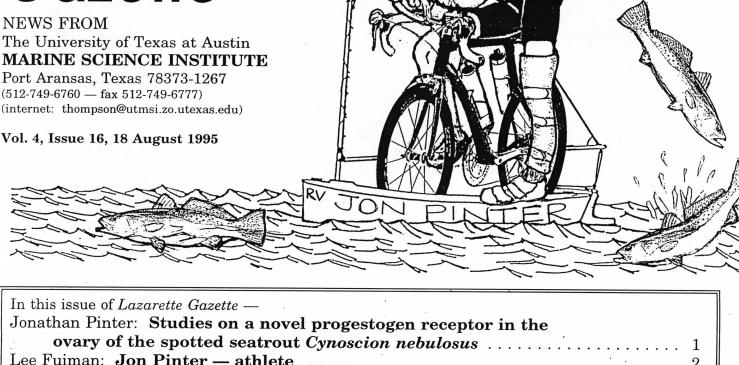


Vol. 4, Issue 16, 18 August 1995



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Abstract

STUDIES ON A NOVEL PROGESTOGEN RECEPTOR IN THE OVARY OF THE SPOTTED SEATROUT CYNOSCION NEBULOSUS

Jonathan Pinter, Ph.D. Supervisor: Peter Thomas

I have characterized a novel progestogen receptor in the spotted seatrout, Cynoscion nebulosus. Nuclear and cytoplasmic binding components have been identified and this receptor has been detected in the ovary, testis, and possibly the liver. This receptor has binding characteristics similar to progesterone receptors in a wide variety of vertebrates, including mammals, birds, amphibians and elasmobranch fishes. One important difference is that the two ligands with the highest affinity for this receptor are the teleost maturation-inducing steroids $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one $(17\alpha,20\beta$ -P) and $17\alpha,20\beta,21$ -trihydroxy-4-pregnen-3-one $(17\alpha,20\beta)$ pregnen-3-one (20β-S). These two compounds are the major progestational steroids in teleost fish as opposed to progesterone, which is utilized by other vertebrates. Unfortunately, the affinity of these MIS's for progesterone receptors (PR) in other vertebrates has not been determined.

The structure of the two MIS's is intermediate between common progestins and glucocorticoids. Interestingly, competition curves with a variety of synthetic agonists and antagonists indicate that this receptor has steroid specificity characteristics intermediate between PR and glucocorticoid receptors (GR). Specifically, addition of a hydroxyl group at the 11 position decreases binding significantly, a characteristic of PR. This receptor also demonstrated characteristics similar to GR's; specifically, the significant decreases in binding caused by long-chain substitutions of the hydroxyl group at the 17 position, and the importance of the hydroxyl groups at positions 20 and 21 in determining binding affinity. Traditionally, the 11 and 17 positions have been heavily substituted to select specifically for binding to PR and not to GR. As a result, none of the synthetic antagonists and only one of the synthetic agonists demonstrated a relative binding affinity greater than 1%. If synthetic compounds were to be developed for this receptor, substitution patterns would have to be devised that did not rely on the 11 or 17 positions. Potential sites include the 6 and 7 positions, as substitutions at these positions did not appear to diminish binding.

Receptor levels were analyzed during gonadal recrudescence in the wild to determine when during ovarian development the receptors might be physiologically active. Due to the differences in gonadal size between males and females, as well as the large range of ovarian weights, receptor levels were calculated on three bases: whole gonad, tissue weight and protein concentration. Analysis of receptor concentrations indicated that receptor levels were unregulated approximately two-fold on a whole gonad basis during ovarian growth. However, receptor levels per mg protein underwent a significant, asymptotic decrease. This was interpreted to indicate a more rapid increase in ovarian growth (vitellogenin uptake) than receptor levels during recrudescence. In contrast, receptor levels in the testis and liver did not appear to be regulated during gonadal growth.

Analysis of receptor regulation in fully-grown oocytes $in\ vitro$ indicated that only the two MIS's are capable of inducing receptor disappearance from the cytosol. Gonadotropin (hCG) and all other steroids tested had no effect on cytoplasmic receptor levels. The time course of disappearance from the cytosol was slightly slower than the association rate for steroid binding. Likewise, the concentration necessary to induce maximal cytosolic displacement was two-fold higher than the K_D . Despite the inability to measure the appearance of the receptor in the nucleus, it is believed that translocation is the mechanism responsible for the observed decrease in cytoplasmic binding sites.

I have confirmed the results of previous investigations indicating that steroid-induced hydration, but not final oocyte maturation, requires a genomic mechanism of action. I have demonstrated that ovulation is also inducible by steroid addition and that RNA and protein synthesis are required for ovulation to occur. This is consistent with studies on goldfish, yellow perch and brook trout, as well as studies in a variety of other vertebrate systems.

In conclusion, a novel progestogen receptor with high affinity for the teleostean MIS's has been characterized in the ovary of the spotted seatrout. This receptor appears to be an evolutionary intermediate between PR and GR in terms of steroid specificity and in its ability to act both as a GR (hydration) and as a PR (ovulation). A final model (Fig. 6.1) can be proposed wherein fully-grown, post-vitellogenic oocytes respond to the surge in maturational gonadotropin (GtH II) by increasing MIS membrane receptors and synthesizing MIS. MIS then acts through the membrane receptor to induce FOM, and through the nuclear receptor to induce the subsequent steps of hydration and ovulation. Further investigations will reveal more information regarding the control of hydration and ovulation, as well as the relationship between the membrane and nuclear receptors.

Students

Jon Pinter was an avid volleyball player, and eagerly accepted an invitation to be in the "big leagues" with our team that played in tournaments in Austin, San Antonio and Houston. In the season opener in Austin everything was going well, but our second match proved to be strong competition. Following an aggressive salvo from the other side, one of our players passed the ball low to the middle of the court. Our setter was not in sight and the ball was inches from the floor. I dived and managed to get my hand under it in time to pop it up a couple of feet. The third player to touch the ball must get it across and Jon took the initiative. With single-minded determination he lunged toward the sinking ball and pooched it to the other side. Unfortunately, in his finely focussed state, he forgot I was still on the floor. As I began to lift my chest from the floor, Jon's feet became entangled in my legs, leaving his center of gravity far in front of his feet and directly over my head. What followed was an athletic corollary of at least two of Sir Isaac Newton's laws of motion: a falling body (Jon's) accelerates at a rate of 9.8 meters per second. I did not have time to calculate the accumulated momentum at the time of impact, but the transfer of momentum to the other body (my head) was complete. My chin smashed into the floor with great force, leaving my head to be assaulted a second time when Jon completed his descent. After the match, Jerry Hoff, another MSI student who was resident in Austin and a spectator, took me to a clinic where I received six stitches in the chin. The incident must not have been significant to Jon or perhaps he thought I was so old and dottering that I'd forgotten it, because he asked me to serve on his doctoral committee. I made little mention of the incident until the time was right. Upon successful defense of his dissertation, Jon was advised that his dissertation would be approved after he made a few changes AND paid all of his bills. At that point I presented him with the bill I received from the medical clinic in Austin years earlier.

—Lee Fuiman

Dr. Paul Dayton of UCSD/Scripps had the questionable honor of being able to hang out with the UTMSI graduate students for most of the weekend as the first victim of the Graduate Student Association (GSA) seminar program. The objective of this program is to allow the MSI graduate students to bring in (and subsequently entertain) someone to speak on a topic important to science, but not usually covered by the normal MSI seminars. Dr. Dayton presented an entertaining and thought-provoking seminar to the MSI on scientific ethics and met with both the faculty and the students. He also got a GSA-led boat tour of many of the local marine habitats, and dined with the graduate students probably more times than he would have liked. The seminar program was a great success, and well attended, and can be repeated, it is hoped, once a year on a somewhat regular basis. Thanks to the Texas Union CoSponsorship Review Board, Terry Whitledge and other members of the MSI faculty for helping the GSA to fund this endeavor. Special thanks to GSA president Sharon Herzka for all her hard work in organizing this event.

—Greg Street

Personnel

Kim Keplar began work as a Laboratory Research Assistant IV August 14. Kim will be working for Tony Amos in doing library searches and compiling data on marine debris in the Corpus Christi Bay area and preparing reports as well as some occasional field work. Kim is from Corpus Christi and is a 1995 graduate of TAMUCC.

Letters to the editor

■ I have been enjoying the LazGaz. I thought it might be time to tell the story of the Raisin Phantom. One night in the late 1960's the Institute was attacked by the Raisin Phantom. There were raisins everywhere. Each typewriter had a raisin right where the keys hit the paper. There were raisins in the test tubes, in crab claws in the hall displays, in drawers, under microscopes, in the sink. There was even a raisin (no doubt an exhibit) under "raisin" in the big unabridged dictionary in the library. When B. J. Copeland was packing to leave the Institute a year or so later more raisins turned up. There were raisins everywhere! Simple detective work found two empty raisin boxes in Colin Nicol's office trash can. Could he have been the phantom. No way. He had no sense of humor. The administration was serious. This dastardly culprit must be found and eliminated. There was talk of expelling the vile graduate student or students who violated every sacred place with raisins. The criminal was never found out. Oh, we all had several suspects, but I don't think that anyone except the principals know who they were. Of course, the administration suspected the students, but we all know that only the administration have master keys. Seems to me that since the vehicle (empty raisin boxes) was traced to the office of a professor and since only professors and senior administrators have master keys, the Raisin Phantom must have been either a professor or senior administrator. I wonder when the peanut phantom will strike. (Richard L. Watson, Ph.D. 1975)

Seminars

■ Dr. Ed Houde, University of Maryland, Striped Bass: cohort dynamics of early life stages in Chesapeake Bay, Friday, August 11.

Cruise Reports & Boat Operations

Cruise #95-648 is continuing. This cruise was reported in the last issue of the LazGaz and is still underway as we near the time of publication. The R/V LONGHORN called on Key West on August 11, taking on fuel, water, groceries, and a few repair and replacement parts both for the ship and research equipment sent from Port Aransas (or purchased and shipped direct). The time in Key West was cut short to allow the R/V LONGHORN to visit the Dry Tortugas. The cruise is continuing on schedule although it was necessary to shut down an engine on the 15th due to a clutch problem. Mostly good weather has prevailed and the R/V LONGHORN expects to be back in Port Aransas on the 21st. (If all goes well, she will have a rebuilt or new gear by the 22nd and be back at sea on another cruise on the 24th.)











Computer Corner

Surplus Computers — A memo from the Associate Vice President and Business Manager, Mike Vandervort, relates that a U. T. System Task Force on University/Public School Collaborations has developed a special program to encourage the transfer of surplus property at no charge to public schools. Public schools are especially in need of computers. We are organizing an annual drive to help supply these needs. It goes on to encourage departments to participate in the drive and describes how to do it. BUT WAIT! MSI is ahead of the U. T. System and the rest of U. T. Austin on this. Last year we (officially and legally) donated surplus computers to the Port Aransas Independent School District. If you have computers (or other equipment) which is surplus to the needs of MSI lets find out first if PAISD needs it.

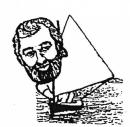
Tony's Tidings...

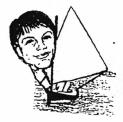
Weather Report for July 31 — August 13

| 31 JUL - 6 AUG 1995 | 5 . MON | TUE WE | D THU | FRI | SAT | SUN MEAN |
|-----------------------|-----------|------------|----------------|------|------|-------------------|
| DATE | 31 | 1 | 2 3 | 4 | 5 | 6 |
| AIR TEMP HIGH | 90.6 | . 89.0 90. | .1 90.6 | 91.0 | 92.1 | . 91.5 90.7 |
| AIR TEMP LOW | | | | | | |
| SEA TEMP LOW | | | | | | |
| RAINFALL TOTAL | | | | | | |
| 7 - 13 AUG 1995 DATE | 7 88.8 | 8 | 9 10 6 90.1 | 11 | 12 | 13 . 86.1 88.4 |

—Andi Wickham











Trip Reports & Travel

Travel ending between August 4 and August 18

→James Kaldy, August 3—5, Port Isabel, Texas, participate in research in Lower Laguna Madre.

→ Ken Dunton, July 17—August 5, Rockville, Maryland; Stony Brook, N.Y., Amherst, Massachusetts; Orono, Maine; to consult with principal investigators on field and laboratory studies planned for February 1996 in Antarctic.



Thanks to Richard Watson for his letter on the Raisin Phantom. Several readers no doubt could write some additional chapters. Please do. I must, however, take exception to Dick's statement concerning the sense of humor of our LazGaz reader in Ribby Lerryn Lostwithiel, retired Professor Emeritus Colin Nicol. Dr. Nicol may write and prove it. And his message shall be humorous but perhaps Geologists may not be treated with great affection. Thanks to Greg Street for writing about Dr. Paul Dayton being able to hang out with the UTMSI graduate students. This eases my mind on something which

has bothered me for several years. When asked what they are going to do, son Ethan has two possible replies: (1) go surfing and (2) hang out, while daughter Jill only has one reply, hang out. I have been somewhat uncomfortable about what hang out means. Now that I know it is something Dr. Dayton does with the graduate students perhaps it is a more respectable activity than I had supposed. Thanks also for help with this issue of the LazGaz to Jon Pinter, Lee Fuiman, Tony Amos, Andi Wickham, Linda Yates, JoAnn Page, Kathy Quade, Lynn Amos, and Patty Webb.

—John Thompson



