REPRODUCTIVE ENDOCRINOPATHY OF EYELESS MUTANT AXOLOTLS

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For the past two or three years we have turned our attention away from the development of tectal connections in eyeless mutants. All our work in this area has been published[1-4].

We have begun to focus on the proximal cause of sterility in the animals, with the optimistic notion that the link between this and eyelessness may be revealed if one could better understand the sterility. (By the way, until there are more alleles at the eyeless locus, it is formally possible that the two phenotypes could be due to closely linked genes that were both inactivated in the spontaneous mutation event. An interesting mutant called ocelliless in Drosophila was missing its ocelli and was also sterile in females. Careful genetic work revealed ocelliless to be two tightly linked genes.)

Our first idea was to stain normal and mutant hypothalamus with antibodies to GnRH. We expected, since van Deusen had shown that the sterility originated in the hypothalamic primordium, to find something wrong with the GnRH fibers. In all animals examined there was a perfectly normal tract of GnRH fibers running along the base of the infundibulum and terminating in the median eminence. Recently, Eagleson and Malacinski have proposed that the cell bodies of some of these axons,especially those anterior to the optic chiasm, are missing or displaced. We got the impression that there were fewer than normal numbers of GnRH

positive fibers in the mutant, although there were still hundreds, so we used RadioImmunoAssays to test the GnRH levels in normals and mutants. Mutants had approximately one third normal levels. Was this because of fewer GnRH cells, or less production, or downregulation due to a defect in the release mechanism, or poor processing of the GnRH peptide? We didn't know.

To help us believe that the defect really was in the GnRH system, we thought we would try to cure eyeless sterility by injecting the animals with synthetic GnRH. But we needed to know appropriate doses before we even started because we had only a limited number of adult eyeless mutants. We therefore decided to try a non-invasive approach like the one they use at the San Diego Zoo for sexing monomorphic rare species of birds and reptiles, and judging reproductive cycles in large mammals. This is simply done by measuring levels of excreted sex steroids. For instance, the ratio of testosterone to estrogen in the urine can be used to sex many species and the daily level of excreted hormones can reveal reproductive cycles or pregnancy.

We first got the technique working in tiger salamanders, collecting water that they had been in for 24 hrs., lyophilizing it, and doing RIAs for testosterone and estrogen. We even found a reasonable dose of GnRH which, when injected intramuscularly, caused a doubling of excreted steroids. The technique also worked for sexing Xenopus. We did the same thing with axolotls, eyeless and normal, and found first that they were no problem to sex: females excreted about 3 times the estrogens that males

did. The levels were the same for both normal animals and eyeless mutants. Unfortunately, neither the normal nor the eyeless animals responded with increased steroidogenic output to daily injections of GnRH, even after 27 days. We were frustrated so we sacrificed the animals at the end of these collections, and found no difference in gonadal weight or maturation between PBS injected and GnRH injected animals. In the normal animals the gonads were mature, in the eyeless they were immature.

We concluded that the non-invasive technique can be a useful tool in studying the reproductive endocrinology of aquatic vertebrates, but it did not get us much closer to understanding the endocrinopathy associated with sterility in the eyeless axolotl. A manuscript with most of these results has been submitted[5].

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5. Korff, G.P., Sarkar, D.K., Bercovitz, A.B. & Harris, W.A. 1987. Measurement of excreted sex steroids in amphibians: A new method for studying reproductive potentials in aquatic vertebrates. (submitted)