

Studies on the Effects of Certain Substances Antagonising the Action of Vitamin a During Limb Regeneration in Anuran Tadpoles.

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Abstract: *Pattern formation is the activity by which embryonic cells form ordered spatial arrangements of differentiated tissues. The ability of cells to sense their relative positions within a limited population of cells and to differentiate with regard to their position has been the subject of intense debate and experimentation. Young anuran tadpoles can regenerate their lost limb elements during metamorphosis And this power gradually declines in proximodistal direction and ultimately disappears in Adult stages.*

Keywords: *Retinoids, antagonizing, anuran tadpoles, Sulfadiazine.*

I. Introduction

Pattern formation is the activity by which embryonic cells form ordered spatial arrangements of differentiated tissues. The ability of cells to sense their relative positions within a limited population of cells and to differentiate with regard to their position has been the subject of intense debate and experimentation. Young anuran tadpoles can regenerate their lost limb elements during metamorphosis And this power gradually declines in proximodistal direction and ultimately disappears in Adult stages. Retinoids are Vitamin A and its natural occurring. They have remarkable affects on various biological activities in animals. They also play an important role in various developmental processes. Vitamin A is also an essential micronutrient necessary for various physiological functions Such as reproduction vision, mucopolysaccharides synthesis and maintainance of epithelial tissue. In the present study an attempt has been made to understand the mode of action of Vitamin A during limb regeneration of an anuran amphibian working on the framework Of following objectives; 1. Stage and mode of treatment dependent effects of retinol palmitate on limb regeneration in anuran tadpoles. 2. To study the effects of some substances which can prevent the effect caused by Rp during limb regeneration.

II. Materials and Methods

The studies of present thesis were carried out on Tadpoles of frog *Rana cyanophlictis* (Schneider). The species identification of frog was done with the help of normal table of development by Taylor and Kollros (1946). The tadpoles of this frog are available in ponds in rainy season. The spawning takes place during early morning hours. Spawn can be seen as a bunch of jelly containing thousands of eggs near The edge of the pond intermingled in aquatic weeds. The eggs were collected in glass beakers filled with water and were transferred to glass troughs having conditioned Water. The spawns were hatched in laboratory aquaria. the tad were reared in plastic troughs they were fed with fresh semi boiled spinach leaves and planktons. Experiments were carried out on stage VII of normal development Average length 2.1 cm Average length of tail 1.4 cm Mouth apparatus : two continuous rows of teeth on upper lip and two continuous rows of teeth on lower lip. Mouth papillae formed on the external margin of lower lip. Rudiments of hind limbs begin to curve slightly. Gills covered by opercular fold. Length of tail increased from 1.2 (stage V) 1.4 cm (stage VII).

Procedure of amputation

In tadpoles limbs were amputated at the level of shank region with a sharp sterile blade under stereoscopic binocular microscope after narcotizing them with 1: 4000 solution of MS222 (Ethyl- m-aminobenzoate- methane sulfonate) obtained from Sandoz Co. The tadpole revive from anesthesia within a few minutes on being transferred to normal rearing medium. The experiment lasted for 15-20 days after amputation. Mode of treatment Suitable doses of rp and sulfadiazine were prepared in water. Dose Screening test were set up by treatment of tadpoles with varying doses of drugs. Retinol Palmitate (Sigma, USA)- 0.4 mg RP dissolved in 1000 ml of water. Sulfadiazine 0.1% solution in normal water. Duration of treatment was 3 days. Min no of 10 tad were used in an experiment. Control group was run separately. Control group was reared in simple water and tadpoles were fed with spinach leaves.

Observations and Results

For experiments concerning studies on limb regeneration stage VII of tadpoles were used. After amputation through shank tad were divided into following experimental groups. Group I - Untreated (control) . In this group tadpoles were amputated through shank level and kept in ordinary water for 15 days. Group II - Tadpoles were treated with 10 IU / ml RP prior to amputation for for 3 days These were then transferred to water for remaining period of experiment. Group III- Tadpoles were treated with 10 IU / ml RP after amputation for for 3 days These were then transferred to water for remaining period of experiment. Group IV - Tadpoles were treated with 10 IU / ml RP after amputation for for 3 days Traumatized at healing site on the 4th day . These were then transferred to water for remaining period of experiment. Group V - Tadpoles were treated with 10 IU / ml RP after amputation for for 3 days and then transferred to 0.1% sulfadiazine for 3 days. These were then transferred to water for remaining period of experiment. Group VI - Tadpoles were treated with 10 IU / ml RP in combination with 0.1% sulfadiazine for 3 days after amputation. These were then transferred to water for remaining period of experiment. Group VI I -Tadpoles were treated with 0.1% sulfadiazine for 3 days after amputation . These were then transferred to water for remaining period of experiment. In various groups used in present study 5 different types of regenerates were observed. 1. Normal type (NT) : These type of limb regenerates showed normal limb Structures having shank ankle and all 5 digits. 2. Incomplete Whole limb (IWL) : These type of limb regenerates showed incomplete whole limbs regenerated at the cut end to the shank, i.e. only part distal to amputation (additional shank, ankle, and all 5 digits). 3. Whole limb regenerates (WLR) : Showed complete whole limb generated at the cut end.Ie thigh, shank, ankle and all 5 digits. 4. Duplicate limb (D L) : These cases showed duplicate limb regenerated at the cut end i.e. 2 limbs having thigh shank and ankle. 5. Abnormal whole limb (A W L) : These types of limb regenerates showed abnormal type of whole limb generated at the amputated site i.e. thigh shank and ankle generated but the number of digits were not normal. 3 or 4 digits were present in most cases. Group I - 100 % showed normal type of regenerates. Group II - These cases showed Normal type of limb regenerates in 90 % cases having shank ankle and all 5 digits. In 10% cases IWL regenerates were observed such cases showed upper shank regions showing swelling rest of the part were ankle and all 5 digits. Group III - 50 % cases showed WLR thigh, shank, ankle and all 5 digits, 30% cases showed IWLR (incomplete whole limb regenerates). having extra shank, ankle and 3 digits instead of 5 or 4 digits, 10% cases showed normal type of regenerates. Group IV - 50% cases showed duplicate limb regenerate ie 2 limbs generated instead of one at the amputated site. In some cases proximal elements of double whole limbs were fused and only ankle elements were separated.50% cases showed incomplete whole limb regenerates. Group V - showed 90 % cases with abnormal whole limb regenerates (AWL) having shank, ankle and 3 digits instead of 5. 10% cases showed whole limb regenerates (WLR). Group VI - 30% cases showed complete whole limb regenerates.(WL).and rest 70% normal type of regenerates (NT). Group VII - All cases showed normal regenerates.

III. Discussion

Sulfadiazine prevents the action of vitamin A (Retinol Palmitate) on limb regeneration Retinol palmitate has been found to cause proximalization of regenerate in anurans following Limb amputation through shank. Tadpoles of anuran and urodele larvae exposed to excess of retinoid during initial phase of regenerate results in Proximodistal duplication of limb elements in regenerates including girdle elements in between stump and regenerate in most cases. In some cases it causes duplication of whole limb. The frequency of occurrence of Proximodistal duplication and multiple whole limb regenerates after retinoid treatment depend on stage of development of tadpole, type of retinoid used, duration of treatment, conc of retinoid etc. (Sharma, 1982; Maden, 1982; Sharma and Niazi 1986). Reviewing properties of retinoids as endogenous components of regenerating limb and tail Maden has suggested that there are sp retinoic acid receptors present in the responding tissue. Maden and Corcoren (1996) have found that effects of retinyl palmitate are prevented by the prior administration of prolactin to Rana tadpoles. Prolactin inhibits the release of thyroid hormone in anurans that suggest that an interaction between retinoids and thyroid hormone perhaps via there respective Receptors Retinoid X receptors (RXR 'S) and Thyroid Hormone Receptors (TR'S) Might involve in transformation of tissue.

IV. Conclusion

Sulfadiazine treatment along with retinol palmitate on limb regeneration caused normalization of regeneration by preventing proximalizing effects of retinol palmitate on limb regeneration. Retinol palmitate delayed metamorphosis of tadpoles. Specific genes sensitive to retinol palmitate are responsible in inducing retinoid based action.

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