Evidence for Corticotropin Releasing Factor (CRF) Synthesis in the Preoptic Nucleus of *Xenopus laevis* Tadpoles

A Preliminary Report Based on Lesion Experiments

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Summary. For the study of the hypothalamo-hypophysial system of *Xenopus laevis* tadpoles, hypothalamic lesions were made by means of the electrocoagulation technique. Lesioning of the ventral region of the preoptic nucleus resulted in a decrease of the number of ACTH cells in the pars distalis of the pituitary gland and in a diminution of the PAS-positive reaction of these cells. In addition, regeneration of the neurosecretory cells of the ventral region of the preoptic nucleus observed 6 weeks after lesioning was accompanied by the reappearance of normal PAS-positive ACTH cells in the pars distalis. It is suggested that the neurosecretory cells of the ventral region of the preoptic nucleus of *Xenopus laevis* tadpoles are related to the ACTH synthesizing cells, probably by producing CRF.

Key words: Hypothalamo-hypophysial system — Preoptic nucleus — Corticotropin Releasing Factor — *Xenopus laevis*, tadpoles — Lesions.

Introduction

In Amphibia the “Gomori-positive” peptidergic preoptic nucleus (PON) can be divided into three regions: the magnocellular rostro-dorsal and caudo-dorsal regions, and the parvocellular ventral region. The rostro-dorsal region is involved in the synthesis of the thyrotropin releasing factor (TRF; Goos, 1969; van Oordt et al., 1972) and the caudo-dorsal region in the production of posterior...
lobe hormones (Notenboom, 1974). The function of the ventral region of the PON is unknown, but may have something to do with the regulation of the adenohypophysis. At any rate, lesioning the ventral and the rostro-dorsal region of the PON results in a marked reduction, if not nearly complete disappearance, of Gomori-positive peptidergic neurosecretory material in the outer zone of the median eminence of *Xenopus laevis* larvae (Notenboom, unpublished results). As in other vertebrates, i.e. mammals (Bock et al., 1969; Brinkmann and Bock, 1973; Watkins et al., 1974; Watkins, 1975) and birds (Péczely and Calas, 1970; Abel et al., 1975), the corticotropin releasing factor (CRF) together with the TRF and the posterior lobe hormones are thought to be represented by Gomori-positive neurosecretory material in the outer and inner zones of the median eminence and are produced by neurosecretory neurons in the hypothalamus. 

It seemed worthwhile to test the possibility of a CRF function of the ventral region of the PON in *Xenopus laevis* tadpoles. For this purpose the effect of lesioning the ventral region of the PON on the periodic acid - Schiff (PAS)-positive cells in the extreme rostral tip of the pars distalis (Kerr, 1966; van Oordt, 1974) was studied in a series of preliminary experiments. In *Xenopus laevis* these cells are known to produce corticotropin (ACTH; Evenett and Larsen, 1970; Doerr-Schott and Dubois, 1972; Doerr-Schott, 1974).

### Material and Methods

**a) Animals.** Tadpoles of *Xenopus laevis* (Daud.) were reared in tapwater at a temperature of 20–22°C; the photoperiod was 12 h light-12 h darkness. The developmental stage was determined with the normal table of Nieuwkoop and Faber (1956). A total number of 75 tadpoles were lesioned at stage 56–57 and killed between 1.5 and 6 weeks after lesioning.

**b) Microsurgical Technique.** Lesioning was carried out according to the technique developed by Mrs. Dr. M.H.I. Dodd (University College of North Wales, Bangor, U.K.) and described by Notenboom (1974). Tadpoles adapted to a white background for at least 2 h were anaesthetized in a MS 222 solution (0.1% in tap water) for a short time. Via the mouth cavity the parasphenoid was pierced with a platinum needle with a tip diameter of 0.1 mm. The tip of the needle was inserted into the ventral hypothalamus just rostral to the optic chiasma, both to the right and to the left medial plane. Electrocoagulation was achieved with a current of 0.7 mAmp for 5 s. Sham operation was carried out by lesioning the optic chiasma or the telencephalon. The exact localization and extension of the lesion was studied with the aid of an atlas based on transverse sections of the diencephalon (Notenboom, 1972). However, tadpoles which showed an arrested metamorphosis (7 animals) were discarded as it could be expected in such animals the lesions were too large and included large parts of the TRF-producing rostro-dorsal region of the PON. The remaining animals (68) completed metamorphosis at a normal rate and were killed at stage 66.

**c) Histological Techniques.** Anaesthetized animals were killed by decapitation and the heads fixed in a slightly modified Bouin’s fluid (Notenboom, 1972). The tissue was decalcified with 5% HNO₃ and after embedding in paraffin, transverse 5 μm sections were made. To study the effect of the lesions upon the preoptic nucleus, the p-rosanilincrotonaldehyde technique (RCA; Bock and Ockenfels, 1970, with p-rosaniline instead of diaminobenzophenon) and the N,N'-diethyl-pseudoisocyanin chloride (PIC) method (Schiebler, 1958; Sterba, 1964) were applied. The oxidation time in the latter technique was reduced to 15 s. For fluorescence microscopical examination of the sections, an excitation filter UG 5 (312–365 nm, Schott) and barrier filters 47 (470–800 nm, Schott) and 55 (326–650 nm, Schott) were used. In order to localize the ACTH cells in the rostral part