Electron Microscopic Observations on the Retino-preoptic Pathway of Rana temporaria L.

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Summary. Electron microscopic observations confirm the presence of optic terminals of retinal origin in the preoptic area of Rana temporaria. After unilateral section of the optic nerve, degenerating axon terminals were observed among the neurosecretory cells of the preoptic nucleus, both ipsilaterally and contralaterally. The retino-preoptic terminals apparently establish axo-dendritic synapses with non-neurosecretory neurons. Many more degenerating fibres were seen in the ipsilateral preoptic nucleus than in the contralateral nucleus. These are presumed to pass through and to cross in the dorsal posterior part of the optic chiasm.

Key words: Retino-preoptic tract — Rana temporaria — Degeneration — Electron microscopy.

Introduction

We have shown, using silver staining of degenerating optic fibres, that in Rana temporaria certain optic fibres pass rostroventrally from the optic tract and run towards the aldehyde-fuchsin positive cells of the preoptic nucleus (Vullings and Kers, 1973). From these experiments no clear demonstration of retinal terminals in the preoptic area was possible. With autoradiographic techniques, however, it could be demonstrated that in Rana a retino-preoptic tract exists. Because of the difference in labelling between the ipsilateral and the contralateral preoptic nucleus after unilateral intraocular injection of tritiated amino acids, it was concluded that this tract is primarily ipsilateral. This agrees with a previous observation (Vullings, 1973) that after unilateral optic nerve section, the secretory activity of the preoptic nucleus was lower on the operated than on the intact side. It also appeared that the retino-preoptic fibres were projected predominantly to the ventral and medial part of the preoptic nucleus (Vullings and Kers, 1973).

Moore, Karapas and Lenn (1971), Hendrickson, Wagoner and Cowan (1972), Meier (1973), Bons (1974) and Hartwig (1974) have presented convincing evidence for the existence of a direct retino-hypothalamic connection in mammals and birds. Graeber and Ebbesson (1972) demonstrated a bilateral projection of optic fibres to the hypothalamus of the shark Negaprion brevirostris. Vanegas and Ebbesson (1973) found a contralateral retino-hypothalamic projection in the teleost Eugerres plumieri. In Ambystoma tigrinum ipsilateral fibres deviating from the optic tract

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and entering the preoptic area seem to cross, while contralateral optic fibres appear to terminate in the preoptic area (Jakway and Riss, 1972).

In this study an attempt is made to obtain supplementary data about the retino-preoptic tract in *Rana* and to determine the nature of the retinal terminals in the preoptic nucleus.

**Material and Methods**

Six intact and 20 unilaterally blinded frogs of the species *Rana temporaria* were used. Unilateral blinding was performed by cutting the left optic nerve. During the survival time the animals were kept at 18°C and in an 18 hours light/6 hours darkness cycle. At different times after the operation (1, 2, 3, 5, 6, 10 days and 2, 3, 4, 5 weeks) two animals were killed by decapitation without being anaesthetized. The brains were immediately fixed in 6% glutaraldehyde in 0.125 M phosphate buffer at pH 7.4. Postfixation with 2% OsO4 in 0.25 M phosphate buffer at pH 7.4 was carried out for 1 hour at 0°C. The 6 unoperated animals, which served as controls, were treated in the same manner.

The brains were embedded in Epon 812 after dehydration through increasing concentrations of acetone or alcohol/propylene oxide. Ultrathin sections were contrasted with uranyl acetate in 70% methanol followed by lead citrate. Pictures were taken with a Zeiss EM9A electron microscope.

**Observations**

From about the sixth day following optic nerve sectioning, axonal terminals showing ultrastructural changes characteristic of degeneration were observed among the neurosecretory cells of the preoptic nucleus. These terminals gradually become more electron dense due both to an increased packing of organelles, particularly of synaptic vesicles, and to an increased opacity of the matrix (Figs. 1a—e). The synaptic contacts formed by these terminals and the postsynaptic elements were discernible up to the 10th day after operation. Although terminals showing early degenerative changes were occasionally observed on the third day after operation, the majority of the terminals showed degenerative changes between the 6th and the 10th day after operation. After this time only darkened profiles not in synaptic contact with postsynaptic elements were observed.

Although in the normal preoptic area many presynaptic endings contain both clear synaptic vesicles and dense core vesicles, the degenerating terminals contain predominantly clear vesicles. Very rarely a few dense core vesicles were observed.

Fig. 1a—i. Degenerative changes in the preoptic nucleus after unilateral section of the optic nerve

Fig. 1a—e. Retinal terminals in different phases of electron dense degeneration. Mitochondria and synaptic vesicles gradually disintegrate into dense clumps. Fig. 1a—c, respectively ×23400, ×22800 and ×21700 from animals killed 6 days after operation. Figs. 1a, c are from the same animal. Fig. 1d, e, respectively ×20000 and ×22800 from animals killed 10 days after operation. Fig. 1a, d, e are from the left (ipsilateral) preoptic nucleus while Fig. 1b, c are from the right (contralateral) nucleus. Arrows point to the sites of synaptic contact

Fig. 1f, g. Degenerating fibres from the ipsilateral preoptic nucleus. The axon is almost completely filled by one or more dark staining inclusions. Five days after operation. Respectively ×51000 and ×27400

Fig. 1h, i. Electron dense profiles engulfed by glia in the left (ipsilateral) preoptic nucleus. Three weeks after operation. Respectively ×24000 and ×20000