isoproterenol was not due to non-specificity of our assay system.

Our experiments do not rule out the possibility that isoproterenol was demethylated to form epinephrine and that it was the activity of this alpha-adrenergic agonist we observed. However, the swiftness of onset of the response to isoproterenol did not differ from that of typical alpha-adrenergic compounds nor is there any evidence from other systems that demethylation is a significant feature in the biological activity of isoproterenol. Thus we can tentatively conclude that the adrenergic receptor in mosquito antennae, that mediates hair erection, differs in at least one aspect from a typical vertebrate alpha-adrenergic receptor in that it is able to accept isoproterenol as an agonist.

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Acetylcholinesterase decrease in the optic lobe after unilateral eye deprivation

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Summary. Unilateral eye enucleation in a teleost and a turtle results in progressive AChE decrease in the optic lobe controlateral to the extirpated eye. The final difference reaches 15% in teleost and more than 20% in turtle. No drastic differences in localization, but a rearrangement of histochemical pattern, due to the degeneration of retinal fibres, is noticed.

Substantial amounts of acetylcholinesterase (AChE, E.C. 3.1.1.7) are usually present in the optic tectum of nonmammalian vertebrates[2]. The enzyme shows a wide laminar distribution in teleosts, while in the series anurans-reptiles-birds it tends to be restricted to the main receptive layer of the tectum, the stratum fibrosum and griseum superficiale[3], where a progressive process of lamination occurs. Chakrabarti et al.[4] demonstrated AChE decrease in the controlateral optic tectum after unilateral visual deprivation in the pigeon. The same effect was noticed by Boell et al.[5] after unilateral enucleation of frog tadpoles. In order to extend knowledge of the relationship between tectal AChE and visual deprivation, we made an experimental study in a teleost and a reptile, trying to evaluate quantitative as well as qualitative aspects by means of biochemical and histochemical methods.

Table 1. AChE concentration in the optic lobes of goldfishes after right eye ablation

Time after operation	Right lobe	Left lobe	p-value
1 week (6)	15.20 ± 0.37	14.73 ± 0.53	> 0.05
2 weeks (6)	16.03 ± 0.55	14.40 ± 0.56	< 0.01
4 weeks (7)	14.53 ± 0.57	11.98 ± 0.54	< 0.01
8 weeks (8)	14.94 ± 0.42	12.80 ± 0.40	< 0.01
12 weeks (7)	15.63 ± 0.51	13.21 ± 0.55	< 0.01
16 weeks (7)	16.42 ± 0.64	13.87 ± 0.68	< 0.01

Value of control animals (6): 15.58 ± 0.76. AChE activity is expressed as μmoles acetylcholine hydrolized/mg dry tissue/h. The results are the mean values of the experiments carried out for each experimental group (numbers in brackets) ± SE. p indicates the degree of significance of the differences recorded in AChE concentration between the right and the left optic lobes, as determined by using the Student t-test.

Materials and methods. About 100 goldfishes (*Carassius auratus*) and 80 aquatic turtles (*Pseudemys scripta*) were used. The animals were kept under standard conditions with a regular alternation of 12 h light and 12 h darkness. The animals were enucleated by surgical removal of the right eye under ether (for turtles) or MS 222 (for teleosts) anaesthesia, and the brains were removed after different survival times; other animals not operated were kept in the same conditions as controls.

For AChE histochemistry the brains were fixed in 10% formolsaline at 4 °C for 3-5 h, cooled and cut in the cryostat. 30 μm sections were processed according to the methods of Gerebtzoff[6] or Karnovsky and Roots[7], adding the selective pseudocholinesterase inhibitor iso-OMPA 2×10^{-5} M during preincubation and incubation (45 min and 90 min at room temperature respectively).

For the quantitative determination of AChE, the right and left optic lobes of each animal were separately weighed and homogenized in 20 mM potassium phosphate buffer at pH 7 (about 10 mg fresh tissue/ml). Samples of the homogenates were used for AChE determination following the colorimetric method of Hestrin[8]. Before addition of substrate, each homogenate was preincubated for 15 min with 2×10^{-5} M iso-OMPA; the incubation with the complete medium was performed for 10-15 min with the same concentration of the pseudocholinesterase inhibitor. The complete medium contained: 2.7 mM acetylcholine iodide; 100 mM NaCl and 20 mM $MgCl_2$. The incubation and preincubation were performed in test-tubes placed in a thermoregulated water bath with a continuous stir; essay temperature was 28 °C for teleosts and 35 °C for turtles. In a

Table 2. AChE concentration in the optic lobes of aquatic turtles after right eye ablation

Time after operation	Right lobe	Left lobe	p-value
2 weeks (6)	11.24 ± 0.23	10.67 ± 0.35	>0.05
3 weeks (6)	11.40 ± 0.65	10.36 ± 0.55	<0.01
4 weeks (6)	10.84 ± 0.45	9.05 ± 0.42	<0.01
8 weeks (6)	11.15 ± 0.38	8.70 ± 0.40	<0.01
12 weeks (6)	11.59 ± 0.98	8.92 ± 0.68	<0.01

Value of control animals (6): 11.14 ± 0.48. Same indications as for table 1.

Comparison between AChE distribution in the right (a) and left (b) optic tectum of a goldfish, 16 weeks after right eye ablation; Gerebtzoff method. A conspicuous reduction in thickness occurs at level of the enzyme-rich bands of the stratum fibrosum and griseum superficiale (sfgs) of the left lobe. In the overhanging stratum opticum (so) there is an almost complete disappearance of the clear band corresponding to the retinal fibre layer. The outermost tectal layer, the stratum marginale (sm), is almost completely deprived of enzyme activity and is only partially seen in the photographs. × 110.

series of experiments both preincubation and incubation were performed in presence of the specific AChE inhibitor BW 284C51 5×10^{-5} M. A series of control and operated animals were used for the determination of the dry weight of the optic lobes heated at 110 °C until constant weight.

Results and discussion. In the goldfish, the differences in the histochemical distribution of AChE are clearly related to the remarkable reduction of the stratum fibrosum and griseum superficiale and the stratum opticum, in the optic tectum controlateral to the extirpated eye. The comparison between the right and the left optic tectum of the same animal 16 weeks after the operation (figure, a, b) shows similar appearance of AChE distribution in the lower part of the tectum. In the stratum fibrosum and griseum superficiale on the contrary the reaction bands of the left tectum are less extended and in closer contact to one another, while the stratum opticum exhibits drastic reduction of the enzymeless band corresponding to the layer of retinal fibres. The modifications in the histochemical picture seem therefore to follow exactly the structural patterns of degenerative process in the tectal lamina[9,10]. In the turtle, differences of the histochemical pattern are difficult to appreciate. Only minor decrease in thickness and reaction intensity occurs in the AChE-rich band of the stratum fibrosum and griseum superficiale, which characterizes the reptilian optic tectum[11,12].

The process of AChE decrease in the optic lobe controlateral to the extirpated eye is shown in its quantitative and temporal terms by tables 1 and 2. No activity was recorded when the homogenates were incubated with 5×10^{-5} M BW 284C51.

Our results provide qualitative as well as quantitative support for the process of AChE decrease in the optic lobe controlateral to the extirpated eye in teleosts and reptiles. The enzyme distribution does not undergo drastic modifications in the tectal lamina; AChE histochemistry only reveals a rearrangement related to the different structural alterations caused by the degeneration of retinal fibres and terminals. The quantitative aspect of AChE decrease is characterized by progressive lowering of enzyme concentration in the controlateral optic lobe with statistically significant final differences. The progressive trend of the process seems characteristic of poikilothermic vertebrates, in contrast with the quick drop in activity reported in the pigeon during the 4th week after operation[4]. The hypothesis of cholinergic transmission between the retinal afferents and the receptive neurons of the tectum seems excluded, at least in birds, by biochemical[13] and electron microscope ultrastructural findings[14]. A possibility to be tested is that AChE decrease is related to the perturbation of intrinsic neural circuits for the modulation of tectal activity. A cholinergic system of similar type was found to operate in the frog optic tectum[15], while the presence of intrinsic control circuits in the optic tectum of several vertebrates is well supported by ultrastructural studies[16-18].

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