Alpha-adrenergic activity of isoproterenol in mosquito antennae

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Summary. Hair erection in isolated antennae of *Anopheles stephensi* males is induced by alpha-adrenergic agonists and blocked by alpha-adrenergic blocking drugs. Hair erection is also induced by a single beta-adrenergic agonist, isoproterenol. The activity of isoproterenol in this system is inhibited by alpha-blockers but not by beta-blockers. These results suggest that the adrenergic receptor for hair erection in mosquito antennae differs from a typical vertebrate alpha-adrenergic receptor.

Males of many species of mosquitoes exhibit a circadian rhythm of erection and recumbence of the long hairs on their antennae. These hairs are usually erected for a period of a few hours about sundown coincident with swarming and mating activity[3]. Erection of the antennal hairs is essential for detection of the female's flight sound, the sole sexual attractant in these mosquitoes[3-5]. Nijhout[6] has recently shown that erection of the antennal hairs is under direct nervous control. Erection of hairs on isolated antennae can be stimulated by alpha-adrenergic agonists and can be blocked by alpha-adrenergic blocking drugs raising the possibility that an alpha-adrenergic agent may be the neurotransmitter in this system. In the present communication we further explore the response of mosquito antennae to adrenergic agonists and antagonists and demonstrate that this system differs from a typical vertebrate alpha-adrenergic system.

Materials and methods. The experiments reported below were performed on males of *Anopheles stephensi*. All experiments were performed between 10.00 and 14.00 h. During this time period all males have recumbent antennal hairs. Males were lightly anesthetized with ether and their antennae were cut off with iridectomy scissors just below their bulbous base in such a way that the proboscis and both antennae were removed as a unit. The proboscis then served as a convenient handle to transfer these antennal sets without damaging the delicate antennal hairs. Antennal sets were floated, base down, on a drop of mosquito saline[7] resting on a siliconized glass slide. Surface tension caused the antennae to float with their long axes perpendicular to the saline surface. The slide was placed in a petri dish lined with moist filter paper to prevent dessication of the preparations. When such antennal sets were transferred to a drop of saline containing also an appropriate concentration of an alpha-adrenergic drug, the proximal hairs of the antennae quickly became erect and were gradually followed by the progressively more distal hairs over a period of 15-60 min. The rate at which hair erection is propagated distally along the shaft of the antenna depends on the drug, the concentration at which it is used and the rate at which it diffuses up the hollow antennal shaft[6]. In view of the fact that antennal hairs are arranged in whorls and that each whorl acts as a unit, it is a simple matter to quantify the response of antennae by estimating the proportion of hair whorls that have become erect at any given time. In the experiments described below a score of 0 indicate that no hairs were erect whereas a score of 10 indicates that all hair whorls were erect. When the mean score of 20 or more antennae was calculated, the response to any one drug solution was quite reproducible. In the experiments described below the status of the antennal hairs was scored 30 min after exposure of isolated antennae to the experimental drug.

Table 1. Response of isolated antennae of *Anopheles stephensi* to various sympathomimetic agents. 3 replicates of 30 antennae were scored on a scale of 0-10 (as described in text), 30 min after exposure to drugs

Compound	Concentration (mM)	Mean score after 30 min
Alpha-adrenergic agonists		
Synephrine	5	10
Epinephrine	5	10
Octopamine	5	10
Phenylephrine	10	10
Norepinephrine	10	8.9
Dopamine	10	4.1
Beta-adrenergic agonists		
Salbutamol	10	0
Terbutaline	10	0
Isoproterenol	10	8.8

Results and discussion. Table 1 lists the response of antennae to various alpha- and beta-adrenergic agonists. Alpha-adrenergic agonists elicited a uniformly positive response[8]. Beta-agonists, salbutanol and terbutaline, elicited no response at all. Surprisingly, isoproterenol, a pure beta-agonist in vertebrates[9], provoked erection of almost all antennal hair whorls in 30 min. Table 2 shows that the activity of isoproterenol was inhibited in the presence of alpha-blockers but not in the presence of beta-blockers. Therefore, isoproterenol is capable of exhibiting alpha-adrenergic activity in this system. It is clear that the effectors for hair erection in these antennae were capable of discriminating accurately between alpha- and beta-adrenergic activity for all other drugs tested. The fact that the activity elicited by isoproterenol was blocked specifically by alpha-blockers indicates that the unusual activity of

Table 2. Effect of alpha- and beta-adrenergic blocking agents on the response of isolated antennae to several sympathomimetic agents. Antennae were preincubated for 30 min on drops of saline containing 10 mM of the blocking agent and were then transferred to saline drops with the same concentration of blocker plus the agonist as indicated. Scores are the mean of 2 replicates of 20-25 antennae each, 30 min after transfer to the 2nd solution

	Adrenergic blockers (10 mM)			
	None	Phenoxybenzamine (alpha-blocker)	Phentolamine (alpha-blocker)	Propranol (beta-blocker)
Epinephrine 1.0 mM	10.0	2.2	0.0	10.0
Octopamine 2.0 mM	8.2	1.3	0.0	8.0
Isoproterenol 4.0 mM	6.1	2.5	0.0	6.0

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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2 We are grateful to the following companies for gifts of drugs used in these studies: Ciba-Geigy (Phentolamine); Astra Pharmaceutical Products, Inc. (Terbutaline); Schering Corp. (Salbutanol), Smith, Kline and French (Phenoxybenzamine). We also wish to thank Dr Louis H. Miller for comments on the manuscript.

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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