

DISTRIBUTION OF ACETYLCHOLINESTERASE IN THE NUCLEUS ISTHMI OF AMPHIBIANS AND REPTILES

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Electrophysiological studies have shown that acetylcholine may be one of the neurotransmitters in the nucleus isthmi (NI) of amphibians and reptiles^[1-3], but little (in amphibians) or nothing (in reptiles) is known about the distribution of its hydrolase, acetylcholinesterase (AChE), within NI. However, abundant AChE is a necessary condition for identifying cholinergic neurons^[4]. Furthermore, we have based ourselves on neuroanatomical and electrophysiological results and suggested that in reptiles the nucleus isthmi pars magnocellularis (Imc) and its parvocellular partner (Ipc) are structurally and functionally independent nuclei^[5]. It could be expected that this will be further supported by histochemical studies. Therefore, we used AChE histochemical^[6] and di-isopropylfluorophosphate (DFP) pharmacohistochemical procedures^[7] to investigate the distribution of AChE in NI of amphibians and reptiles.

I. MATERIAL AND METHODS

Experimental animals were adult frogs *Rana nigromaculata* with 4–7 cm body length, and lizards *Gekko gekko* 11–14 cm long (from the tip of the nose to the cloaca). Seven frogs and four gekkoes were anesthetized with ether, and transcardiacally perfused with physiological saline followed by 10% formalin in phosphate buffer (pH 7.0). The brains were removed and post-fixed for 3–4 h, transferred into 30% sucrose phosphate buffer overnight, then frozen-sectioned at 40–80 μm , and stained according to Karnovsky-Roots^[8].

Additional 8 frogs were intramuscularly injected with 1.1–1.8 mg DFP in arachis oil per gram of body weight; they survived for 3–7 h at room temperature; 5 gekkoes were killed 7–9 h after injection of 12–22 mg DFP/kg body weight. Histological and histochemical procedures for AChE staining were the same as mentioned above.

II. RESULTS

1. AChE Pattern of the Frog Nucleus Isthmi

AChE staining pattern of NI cross sections could be differentiated into 3 regions

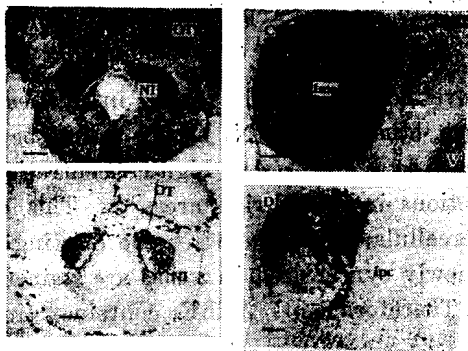


Fig. 1. Microphotographs of transverse sections of midbrain in frogs (A, B) and gekkos (C,D), showing AChE histochemistry of NI (upper row) and its DFP-pharmacohistochemical staining pattern (lower row). Abbreviations Imc, Ipc and NI see text; OT, the optic tectum, Scale: bars, 300 μ m in A, B, and 100 μ m in C, D.

(Fig. 1): The heavily stained dorsolateral region is crescent-shaped and capped on the lightly stained medioventral region, and there is a narrow band between them. The more rostralward a section is, the more conspicuous the band; at the rostral pole it becomes a gap separating the nucleus into 2 divisions evenly stained. The caudal nucleus is lightly and evenly stained as a whole. In all sections, AChE-stained NI is well-defined.

In DFP-pretreated frogs, somas of NI cells are heavily stained. Some cells contain stained granules, others are evenly stained. It appears that all of NI cells are rich in AChE. In some sections, there are several AChE-containing neurons scattered in the posterodorsal tegmental nucleus and the posteroventral tegmental nucleus. However, the boundary line of this nucleus is clear-cut (Fig. 1B).

2. AChE Pattern of the Gekko Nucleus Isthmi

Among the two subdivisions of the gekko NI, Imc is heavily and evenly stained and well-bordered; Ipc is not colored at all. However, the mesencephalic tract of the Vth nerve passing by medially to Ipc is stained (Fig. 1C).

In DFP-pretreated gekkos, Imc cells are stained while Ipc is unseen, and the mesencephalic tract disappeared. It seems that all of Imc cells contain abundant AChE (Fig. 1D). Within Imc, the rostral medulla is sparse-celled while the caudal medulla relatively dense-celled, and there is a cell-free area in the mid-ventral medulla. Moreover, somas in the nucleus profundus mesencephali (NPM) also contain AChE.

III. DISCUSSION

This paper has for the first time studied AChE histochemistry of the reptilian NI and indicated by using pharmacohistochemical procedures that NI neurons in amphib-

ians and reptiles contain high levels of AChE. This lends considerable support to the notion that the amphibian NI and reptilian Imc are well-defined mesencephalic nuclei^[5,8], and Imc and Ipc are structurally and functionally independent nuclei^[5].

It appears that the evenness of AChE staining intensities is not an artifact caused by diffusion of this enzyme, otherwise the NI border cannot be so clear. Therefore, heavily stained regions in the frog NI represent high concentrations of AChE. There may be dense neuronal connections in AChE-rich areas^[9]. This enzyme is distributed in somas, processes and intercellular matrix. After the existing AChE is irreversibly inhibited by DFP, AChE newly synthesized in somas are stained before it has been transported to the processes. Therefore, only AChE contained in somas can be demonstrated^[7].

The presence of high concentrations of AChE within NI also provides some evidence for the previous electrophysiological results that acetylcholine may be one of neurotransmitters in the nucleus^[1-3]. Great reduction in AChE activity in the contralateral NI after enucleation of the frog eye^[10] perhaps implies that in frogs, as in pigeons^[11], tecto-isthmic fibers are cholinergic. It is known that high levels of AChE are necessary conditions for identifying cholinergic neurons^[4], and the frog NI is an important source of cholinergic activity in the tectum^[12]. From these, it could be suggested that at least a part of isthmo-tectal fibers are cholinergic. This could be the case with the reptilian tectum-Imc system, even though with the tectum \rightleftharpoons NPM \rightleftharpoons Imc pathways.

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