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In vitro evidence and age-related changes for nicotinic but not muscarinic acetylcholine receptors in the central nervous system of *Sepia officinalis*

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Abstract

Binding putative muscarinic ([^{3}H]-NMS and [^{3}H]-QNB) or nicotinic ([^{3}H]-cytisine) acetylcholine receptors was quantitatively studied through the use of in vitro binding experiments on either membrane preparations or brain sections of juvenile (3 months), mature (15 months) or senescent (23 months) cuttlefish. No specific binding could be detected with muscarinic receptor ligands under any of the experimental conditions employed (ligand concentrations, buffers, ionic charges, types of tissue, i.e., brain sections or membrane preparations). On the other hand, [^{3}H]-cytisine demonstrated a specific and saturable binding with a single class of high affinity binding sites (K_d of 2.6–34.6 nM; B_{max} of 128–1682 fmol/mg tissue equivalent, depending on the central structure). This binding was found to be heterogeneous throughout the central regions (optic lobe > pedal lobe; superior frontal lobe > . . . precommissural lobe; vertical lobe > . . . anterior basal lobe; subvertical lobe; inferior frontal lobe; median basal lobe). These results question the existence of muscarinic-like receptors in the cuttlefish brain, or at least of a pharmacological dissimilarity from vertebrate muscarinic receptors. In contrast, nicotinic-like receptors are widely present; interestingly, their density was found to be significantly reduced in most nervous central lobes of senescent cuttlefish when compared with mature animals. The most significant decrease (−71%) was found in the anterior part of the superior frontal lobe, which is involved in visual learning; this might be related to the changes, previously demonstrated, in cholinergic neurons in this lobe in the course of aging.

Keywords: Acetylcholine receptors; Central nervous system; Age; Mollusks; Cephalopods; Autoradiography

Acetylcholine of the central nervous system has been studied in cephalopods since 1935, when Bacq and Mazza found evidence for acetylcholine and its catabolic enzyme, acetylcholinesterase, in octopus nervous ganglia [2]. The central nervous system (CNS) of cephalopods appeared to contain large amounts of acetylcholine [3,14,20]. Indeed, quantification of acetylcholine synthesis and catabolism activity in several CNS structures showed a heterogeneous distribution of these enzymes throughout the cuttlefish CNS and a large decrease in these activities with aging [4]. However, very few studies have focused their aims on the characterization of acetylcholine receptors in cephalopods.

Two major classes of receptors for acetylcholine have been found in the CNS of vertebrates: these are the muscarinic type, mAChR, seven transmembrane domains G-protein-coupled receptors activated by muscarine (M1–M5 subtypes), and the nicotinic type, nAChR, pentameric cation-gated receptors activated by nicotine (skeletal muscle and neuronal subtypes). Both muscarinic and nicotinic receptors have been found in the CNS of invertebrates, i.e., in insects [12,27] and marine mollusks [18,25,26].

In cephalopods, binding, electrophysiological and behavioral studies have revealed different types of acetylcholine receptors. The presence of mAChR in the CNS was suggested

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by (i) [^{3}H]-atropine binding on membrane fragments from optic lobes of squid [17] and (ii) by the demonstration that injections of the mAChR antagonist scopolamine impaired long-term memory in octopus [13]. There is also evidence for peripheral mAChR; the suggestion of Hanlon et al. [16] that mAChR may be involved in iridophore control has recently been confirmed by Mäthger et al. [22]. Central nicotinic-like receptors have also been characterized in membrane preparations of squid CNS; binding data showed that the neuronal acetylcholine receptors of squid optic lobes are pharmacologically similar to the nicotinic receptor of vertebrate skeletal muscle [10,17,19]. Two binding site subtypes (one more sensitive to alpha-bungarotoxin and the other more sensitive to agonists) have been revealed [6]. Functional studies using cholinergic agents also suggest the presence of nicotinic receptors in the CNS of cuttlefish. In the optic lobe, spontaneous excitatory postsynaptic currents are blocked by a nicotinic receptor antagonist [9]. Moreover, when injected into central nervous structures involved in motor control, nicotinic receptor agonists and antagonists, respectively, induced or blocked specific stages of predatory behavior [15].

The present study aimed to characterize and localize the cholinergic receptors in the cuttlefish CNS. The binding of radiolabeled ligands, specific either to the muscarinic type or to the neuronal nicotinic type, was quantified either on CNS sections through in vitro autoradiography or on isolated membranes. The short life span of the cuttlefish and the knowledge of its life cycle in the English Channel allowed us to study how aging affects these receptors.

The cephalopod brain consists of lobes aggregated into a central mass organized around the esophagus and protected by a cranial cartilage. The central mass is made up of the supra-, peri- and subesophageal parts, and consists of 38 lobes flanked laterally by two massive optic lobes [5,31,32,33,34]. The central mass lobes (Fig. 1C) all follow the same pattern: a central neuropile (neuron fiber area) and a surrounding cortex (cell bodies). The optic lobes comprise an outer cortex and a central medulla (Fig. 1F). As shown in Fig. 1H, the cortex is composed mainly of two layers of cell somata (the outer and inner granule cell layers) separated by a complex plexiform zone. The large medulla area is less regularly structured, consisting of numerous clusters of cell bodies separated by areas of nerve fibers and synaptic areas.

The cuttlefish, *Sepia officinalis*, used in this study were trawled in coastal waters of the English Channel off Ouistreham in northern France. The life cycle of *S. officinalis* is

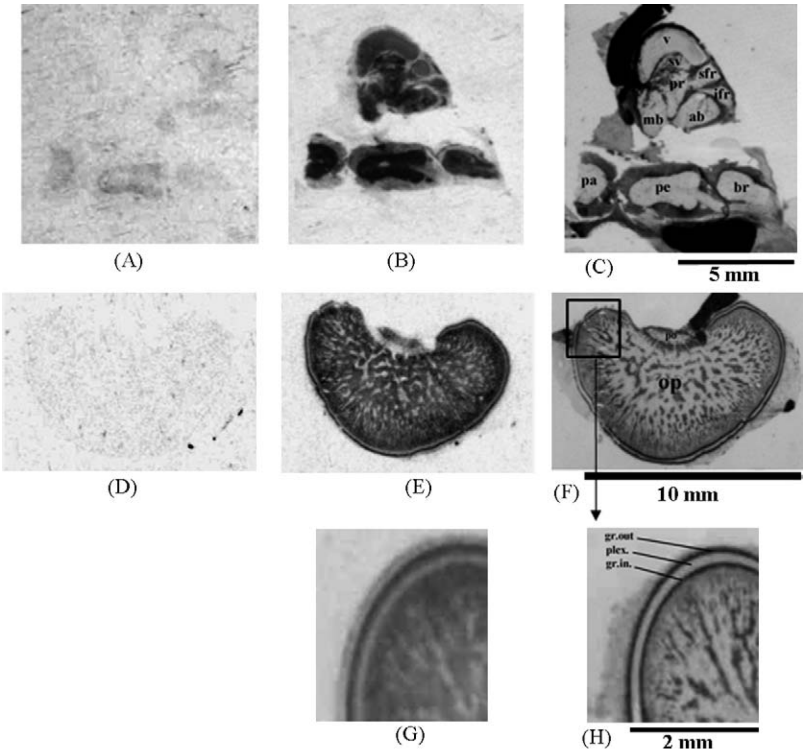

Fig. 1. Localization of [^{3}H]-cytisine binding sites in the central nervous system of *S. officinalis*. Pictures of sagittal sections of a central mass (A–C) of horizontal sections of an optic lobe (D–F) and detail of cortical layers in the optic lobe (G and H) of a subadult cuttlefish. In the first column, (A) and (D) are autoradiograms of the non-specific binding (3 nM [^{3}H]-cytisine, 10 μM nicotine). B, E and G are autoradiograms obtained in total binding conditions (3 nM [^{3}H]-cytisine). In the last column, C, F and H sections stained by Blue Nil after film exposition and used for localization of the binding sites. *Abbreviations for the different lobes*: ab: anterior basal; br: brachial; ifr: inferior frontal; mb: median basal; op: optic lobe; sfr: superior frontal; pa: palliovisceral; pe: pedal; po: peduncle-olfactory; pr: precommissural; sv: subvertical; v: vertical. For the optic lobe cortex: gr.out.: outer granular layer; plex.: plexiform layer; gr.in.: inner granular layer.