

PHARMACOLOGICAL AND HISTOCHEMICAL EXAMINATION OF THE VENA CEPHALICA OF *SEPIA OFFICINALIS* L. (CEPHALOPODA)

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Abstract: This study reveals results on the mechanisms modulating peristalsis of the Vena cephalica in *Sepia officinalis* (L.) (Cephalopoda). The pharmacological data provide evidence for two antagonistic receptor systems in the Vena cephalica. Cholinergic transmitters, like acetylcholine and nicotine, have a positive effect on peristalsis of the Vena cephalica whereas aminergic transmitters cause a standstill of peristalsis in the Vena cephalica. Histochemical and immunohistochemical tests confirm the presence of a cholinergic transmitter system. The regulation of peristalsis in the Vena cephalica resembles closely the neuroregulation of the gastrointestinal tract of other invertebrates and vertebrates than to other circulatory organs of *Sepia officinalis*.

Key words: Acetylcholine, cephalopoda, cholinergic receptor system, circulation system, nicotine, peristalsis, *Sepia officinalis*, Vena cephalica

INTRODUCTION

Functionally the circulation system of the dibranchiate cephalopods is subdivided into a systemic and respiratory part (Fig. 1) (Tompsett, 1939; Schipp, 1987a). Like in vertebrates at cephalopods the thick-walled arteries enable a high-pressure circulation by an air vessel-function, (Shadwick and Nilsson, 1990). In cephalopods, however, the reflux of deoxygenated blood towards the respiratory system is effected by large propulsive veins (de Wilde, 1956; Schipp, 1987a). Especially the Vena cephalica (CV) of *Sepia officinalis* produces powerful peristaltic waves posteriorly along this vein, driving the blood from the head region towards the Venae cavae (Tompsett, 1939).

The wall of this vein is composed of four-layers (Fig. 2) (Smith, 1962; Barber, 1966; Schipp and Schäfer, 1969; Schipp, 1987b). The peristalsis of the Vena cephalica in *Sepia officinalis* is based on two muscle systems of different function (Alexandrowicz, 1965). The periadventitial, longitudinal muscle layer (PLM) of the Tunica periadventitia effects cranial to caudal contraction waves, while the circular muscle fibres of the Tunica media (cMF) are responsible for peristaltic contractions of the vessel (Schipp, 1987b).

In addition, the Vena cephalica takes part in regulating the hemodynamics of the systemic and branchial hearts, the bulbus cordis branchialis, the arterial system and the contractile branchial gill artery. Initial evidence for this is provided by studies demonstrating that this organ together with nerve fibres form a neuronal plexus (NSV-system = Neurosecretory system of the Vena cephalica) which tends to produce transmitters such as hormones, FMRFamide and other cardioexcitatory peptides into the circulatory system (Alexandrowicz, 1964; Alexandrowicz, 1965; Martin, 1968; Young, 1969; Young, 1971; Martin and Voigt, 1987).

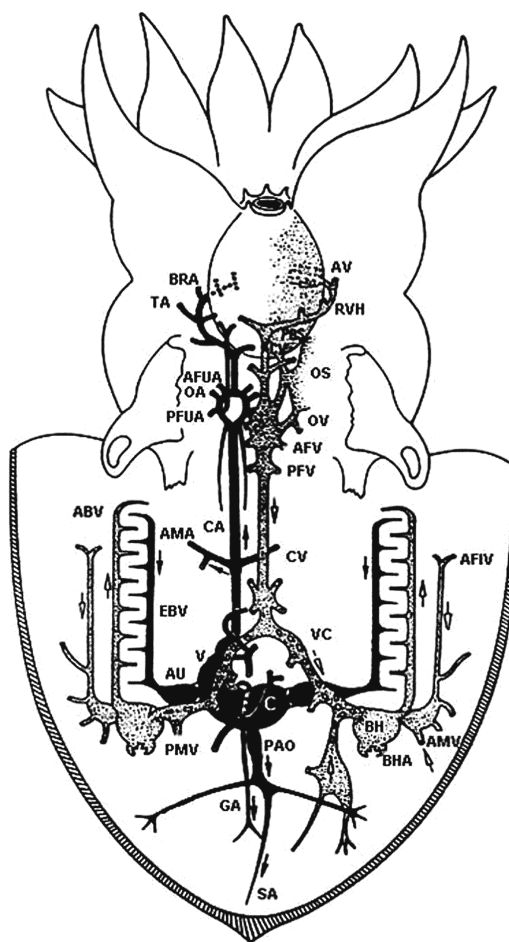


Fig. 1 Ventral view of the circulatory system of *Sepia officinalis*. The arterial circulation system is shown black and the venous system grey. Explanations to anatomy and function of the blood circulation system and abbreviations can be found in Schipp (1987a).

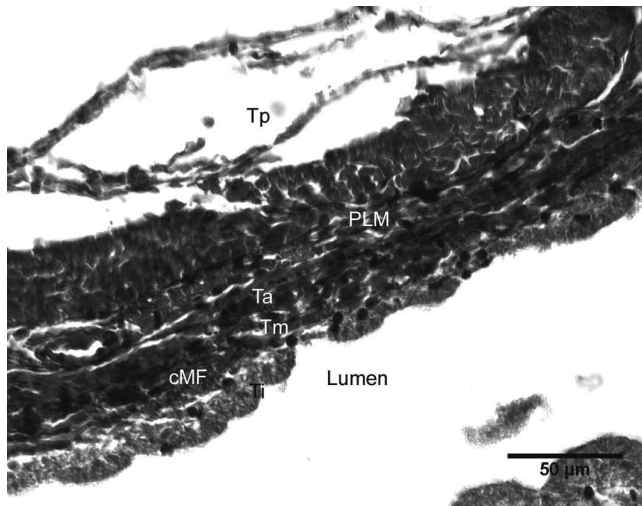


Fig. 2 Cross section through the wall of the ventral vessel. The vein is composed by four layers (Tunica intima (Ti), Tunica media (Tm), Tunica adventitia (Ta) and Tunica periadventitia (Tp). Two muscle systems, the periadventitial longitudinal muscle layer (PLM) and the circular muscle fibres (cMF), are responsible for peristaltic contractions of the vessel. AZAN

This neurosecretory system of the Vena cephalica is comparable with the adrenaline system in vertebrates (Wells, 1983).

The present study focuses on the nature of the cholinergic and aminergic mechanisms modulating the peristalsis of the Vena cephalica in *Sepia officinalis*. Histochemical and immunohistochemical tests will provide information on the nature of the cholinergic mechanisms.

MATERIAL AND METHODS

Animals were anaesthetised, opened from the ventral side and the Vena cephalica, including the cephalic bulb and attached visceral nerves, were dissected out. Tissue and nerves were removed from the vein while myogenic components like the periadventitial muscle layer were not removed from preparations. In the isolated vein an afferent and an efferent cannula were inserted, closed by a ligature and all existent flows towards the vein were tied up. The preparations were removed freshly without any time interval and fixed horizontally in an organ bath (Fig. 3).

The two-walled water-jacketed organ bath was filled with physiological solution (filtered seawater with 0.16% glucose, maintained at 18–19 °C, pH 8.5). The pharmacological solutions were perfused over a four-tap-system into the vein or through the bypass. These solutions, by peristalsis, evoked pressure-signals that were registered by a pressure transducer, amplified and recorded by a pen recorder. This experimental set-up has already been successfully employed in other experiments (Schuck, 1988; Agnisola and Houlihan, 1994). All veins were perfused orthograde under constant conditions (preload pressure 10 cm water pillar; afterload pressure 1 cm water pillar) initially with physiological solution in order to obtain a solid starting condition and to wash out the remaining hemolymph.

DRUG APPLICATION

The drugs (acetylcholine (ACh), adrenaline, dimethylphenylpiperazinium iodide (DMPP), d-Tubocurarin isoprenaline, nicotine, noradrenaline, muscarine, tetraethylammonium and α -BTX) were freshly dissolved in physiological solution and perfused cumulatively through the vein at increasing concentrations (10^{-9} mol/l to 5×10^{-5} mol/l in tenth power steps). For every series of concentrations in the perfusions, an actogram with physiological solution only was first recorded; this was related to a specific transmitter effect. From this recorded curve the amplitude and frequency were determined and computed in the calculation as the “0”-value in the dose-response curves.

IMMUNHISTOCHEMISTRY

Isolated vein preparations from adult specimen were freshly removed, fixed in Bouin solution and 4% saline formalin for 24 h. at RT, washed in 70% Ethanol or running water, embedded in paraffin, cut in 7 μ m sections and mounted on slides. For fluorescence histochemical examinations, preparations were embedded in Tissue Tec[®] (Sakura Inc.) and frozen in liquid nitrogen.

The investigations provided proof of Acetylcholinesterase (E. C. Nr. 3.1.1.7) at the Vena cephalica (Karnovsky and Roots, 1964).

For immunohistochemical marking, a polyclonal antibody (Santa Cruz Biotechnology SC-5544) was used, binding specifically on a protein of the $\alpha 7$ subunit at nicotinic acetylcholine receptors (Santa Cruz Biotechnology Inc., 2001).

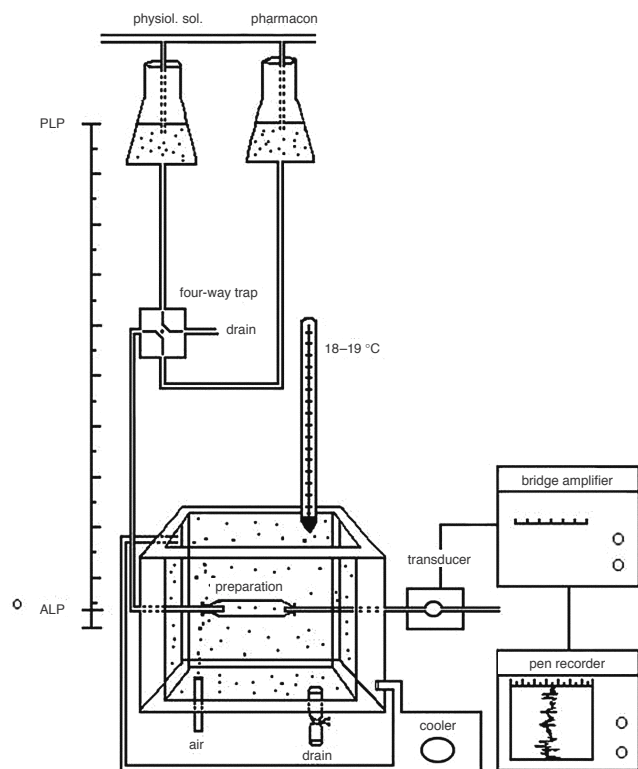


Fig. 3 This scheme represents the set-up for the pharmacological experiments. The preload Pressure (PLP) was adjust to 10 cm and the afterload Pressure (ALP) to 1 cm opposite preparation level. Scheme from Schuck (1988) modified by von Byern.

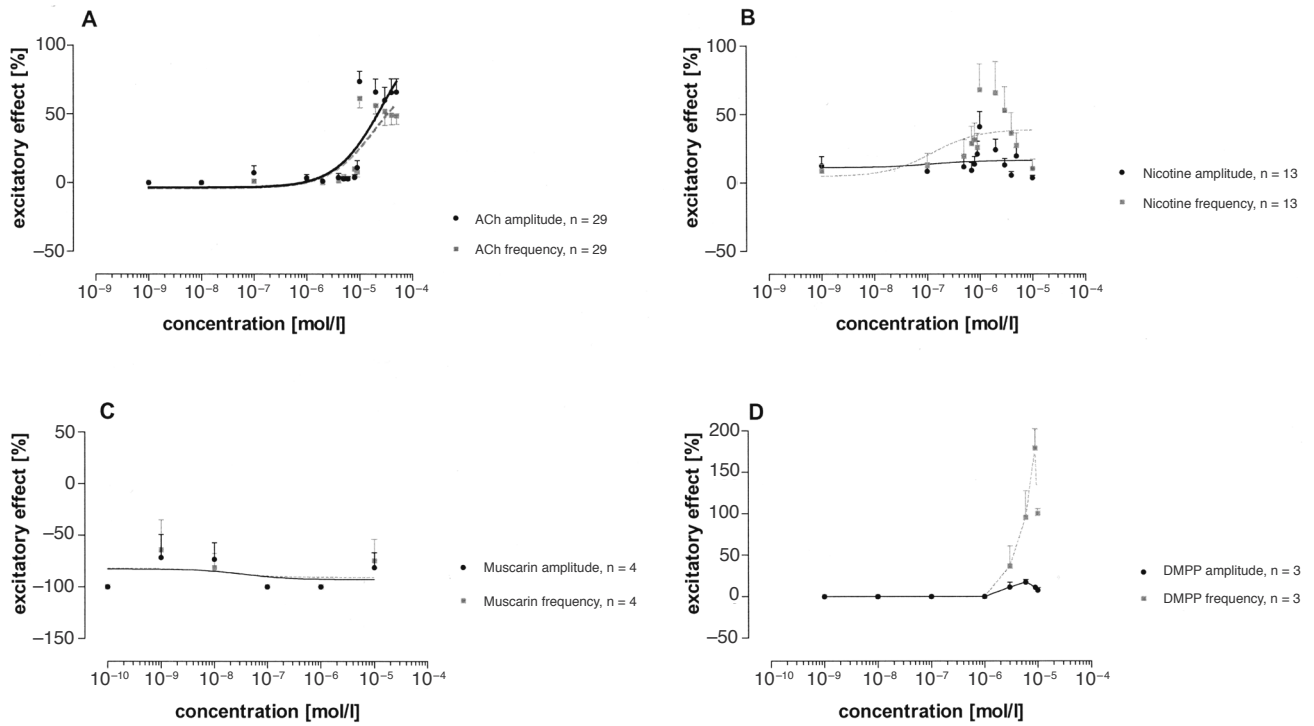


Fig. 4. Binomial concentration-response curve of a) acetylcholine (ACh), b) nicotine, c) muscarine and d). DMPP on the amplitude and frequency of the perfused Vena cephalica. Values are expressed as mean \pm SD.

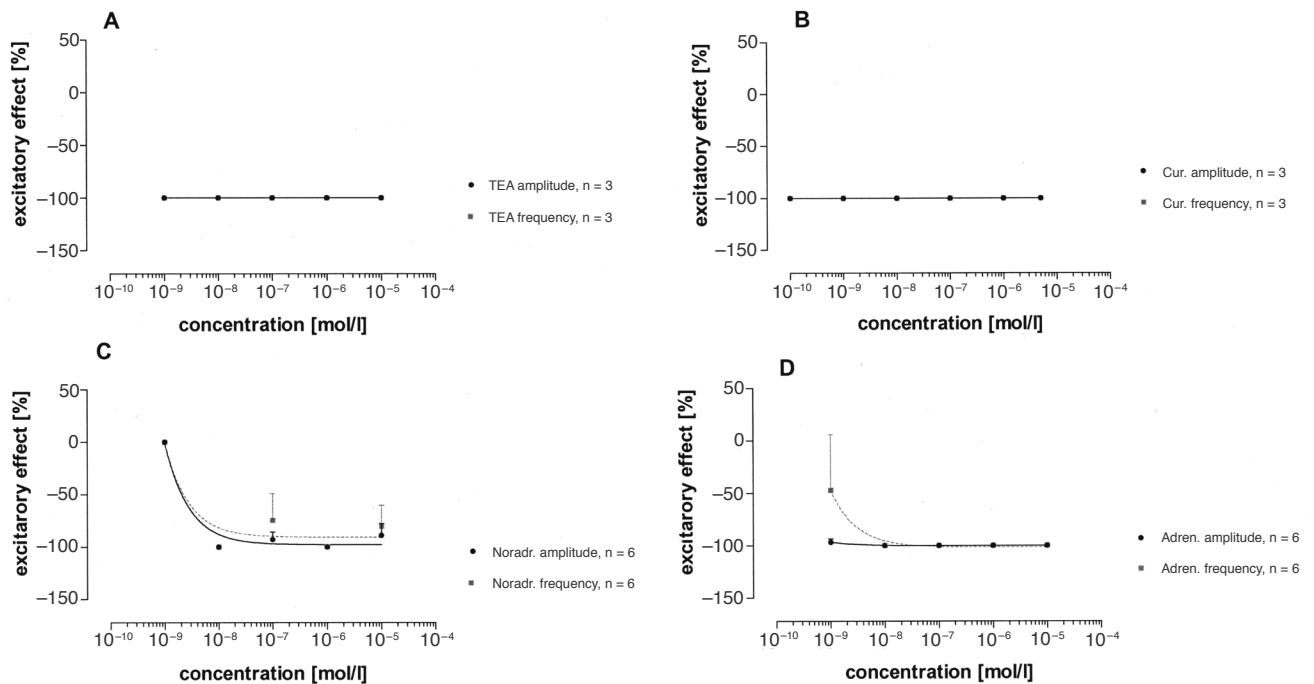


Fig. 5 Binomial concentration-response curve of a) TEA, b) d-Tubocurarin (Cur.), c) noradrenaline (Noradr.), d) adrenaline (Adren.) on the amplitude and frequency of the perfused Vena cephalica. Values are expressed as mean \pm SD.

For fluorescence marking of FITC with α -Bungarotoxin (Sigma-Aldrich T-9641), preparations were first perfused in the organ-bath with acetylcholine (concentration 5×10^{-5} M) for 20 min. The isolated vessels were then fixed for 1 hour in the dark in 10^{-7} Mol FITC- α -Bungarotoxin. Cryostat sections of the samples were photographed (wave length 355–425 nm, barrier filter 460 nm) with a fluorescence microscope. For controls, only FITC was used.

RESULTS

The physiological results of denervated vein preparations show that a myogenic automatism is responsible for the peristalsis of the Vena cephalica of *Sepia officinalis*.

The pharmacological data presented here provide evidence for two antagonistic receptor systems in the Vena cephalica. Cholinergic transmitter, like Acetylcholine and

nicotine, the nicotinic acetylcholine receptor agonist dimethylphenylpiperazinium iodide (DMPP) induces a positive inotropic and chronotropic effect on Vena cephalica peristalsis (Fig. 4). Muscarine and cholinergic antagonists like Tetraethylammonium (TEA), d-Tubocurarin and α -Bungarotoxin (α -BTX) reversibly block the peristalsis in the Vena cephalica. Also aminergic transmitter (adrenaline, isoprenaline, noradrenaline) causes a reversible peristalsis standstill (Fig. 5).

The present immunohistochemical data provide evidence for Acetylcholinesterase (AChE) in fibrous structures of the longitudinal muscle of the Tunica periadventitia and Nervus visceralis, indicating a cholinergic transmitter system (Fig. 6).

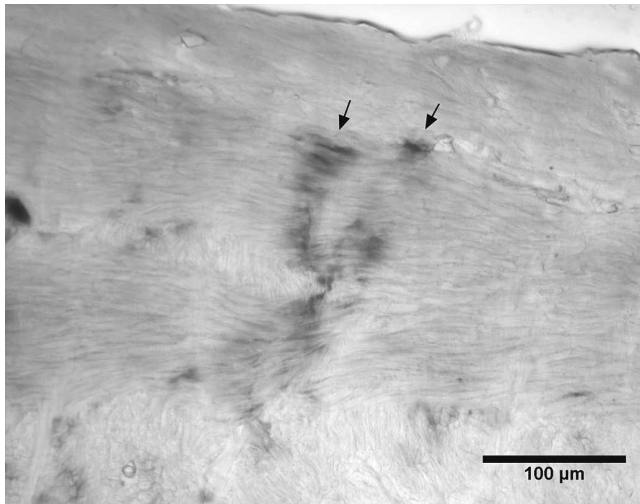


Fig 6 Hatchett-Brown enzyme reactions of AChE (arrows) can be found at muscle fibres of longitudinal muscle layer of the Tunica periadventitia.

The α 7-subunit of a nicotinic ACh receptor subunit (sc-5544) of vertebrates (Santa Cruz Biotechnology Inc., 2001) show clear brown precipitations in the longitudinal muscles of the Tunica periadventitia and Nervus visceralis in the Vena cephalica of *Sepia officinalis* (Fig. 7 and 8).

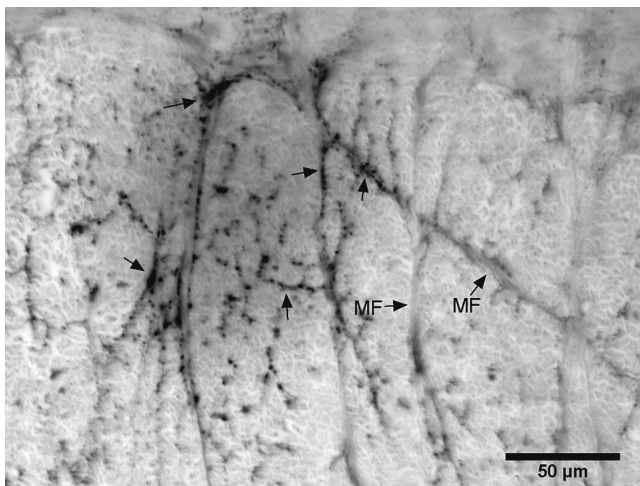


Fig. 7 Immunohistochemical precipitations (arrows) of the antibody against the α 7 subunit of nicotinic acetylcholine receptor yield positive results at muscle fibres (MF) of the Tunica periadventitia.

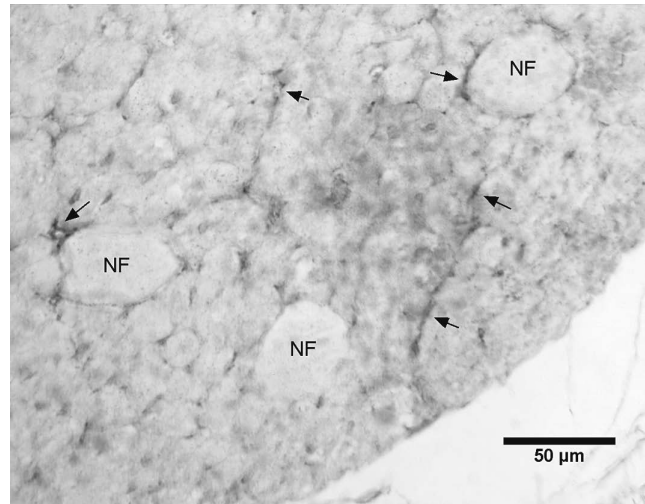


Fig. 8 Immunohistochemical precipitations of the antibody (arrows) around nerve fibres (NF) of the visceral nerve.

The used toxin FITC α -Bungarotoxin allows no clear conclusions about the presence of muscular nicotinic receptors in the Vena cephalica of *Sepia officinalis*.

DISCUSSION

Pharmacological studies on the physiology of the systemic heart, the arterial system and the branchial hearts point at an antagonistic transmitter system with cholinergic (inhibitory) and monoaminergic (excitatory) neuronal mechanisms (Schipf, Schmidt, and Fiedler, 1986; Kling, 1987; Kling and Schipp, 1987; Schipp, Jakobs, and Fiedler, 1991; Fiedler, 1992; Schipp and Fiedler, 1994; Gebauer and Versen, 1998; Versen et al., 1999; Lehr and Schipp, 2004a; Lehr and Schipp, 2004b).

The present pharmacological data provide evidence for a functionally different regulation of the Vena cephalica different from the remaining circulatory system of *Sepia officinalis*. Cholinergic transmitters, like acetylcholine, nicotine and the nAChR agonist DMPP, excite the peristalsis while aminergic transmitter (adrenaline, isoprenaline, noradrenaline) and muscarine cause a reversible standstill of peristalsis.

The regulation mechanisms of Vena cephalica peristalsis are apparently similar to the peristaltic waves of the gastrointestinal tract, which is regulated by similar excitatory cholinergic and inhibitory aminergic mechanisms (Johnson et al., 1987; Furness and Costa, 1987). The immunohistochemical results successfully demonstrate that a cholinergic transmitter system or acetylcholine-catabolic system takes part in peristalsis of the Vena cephalica in *Sepia officinalis*.

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