FUNCTIONAL MORPHOLOGICAL ASPECTS OF THE CEPHALIC AORTA OF SEPIA OFFICINALIS L. (Mollusca : Cephalopoda)

R Schipp, B Fronk, M Kopsch, A Polenz

VIE MILIEU, 1997, 47 (2) : 111-116

FUNCTIONAL MORPHOLOGICAL ASPECTS OF THE CEPHALIC AORTA OF *SEPIA OFFICINALIS* L. (Mollusca : Cephalopoda)

R. SCHIPP*, B. FRONK, M. KOPSCH, A. POLENZ

** Correspondent address : Institut für Allgemeine und Spezielle Zoologie, Justus-Liebig-Universität Giessen, Stephanstrasse 24, 35390 Giessen, Germany*
Laboratoire d'Océanographie Biologique, 33120 Arcachon, France
Observatoire Océanologique, 66650 Banyuls-sur-Mer, France

CEPHALOPODA
SEPIA
AORTA
CYTOLOGY
INNERVATION

ABSTRACT. – Cytological differentiations of the cephalic aorta of *Sepia officinalis* L. were analysed by LM and TEM. The origin and ultrastructure of the elastic fibres are demonstrated. The highly specialised cross striated muscle cells of the T. media with their particular Z-patch system and close interdigitations with extended areas of gap junctions represent a functional syncytium. Only the peripheral layer of muscle cells of the T.m. is innervated. The histo- und immunhistochemical findings confirm earlier pharmacological results suggesting that the vessel tonus is under a multiple, i.e. cholin-, catecholamin- and peptidergic neurocontrol. The role of 5-HT as a putative neurotransmitter in this mechanism is not yet clarified.

CEPHALOPODA
SEPIA
AORTE
CYTOLOGIE
INNERVATION

RÉSUMÉ. – Les différenciations cytologiques de l'aorte céphalique de *Sepia officinalis* L. sont analysées par microscopie photonique et électronique. L'origine et l'ultrastructure des fibres élastiques sont mises en évidence. Les cellules musculaires striées très spécialisées de la Tunica media avec un système particulier de z-patches et des jonctions gap étendues dans les domaines d'interdigitations cellulaires étroites représentent un syncytium fonctionnel. Seule, la couche musculaire périphérique de la T.m. est innervée. Les observations histo- et immunohistochimiques confirment des résultats pharmacologiques anciens indiquant que le tonus du vaisseau est sous contrôle multiple, c'est-à-dire cholin-, catécholamin- et peptidergique. Le rôle de 5-HT comme neurotransmetteur éventuel dans ce mécanisme n'est pas encore clairement établi.

INTRODUCTION

The cephalic aorta of coleoid cephalopods represents a good example of a high pressure "Windkessel" vessel in invertebrates that shows structural and functional properties corresponding to those of larger vertebrate arteries (Bourne 1982; Shadwick & Gosline 1983, 1985 a,b; Schipp 1987).

In comparison to the cephalic aorta of nautiloids the coleoid aorta has a thick wall, but a relatively low volume and a low extension coefficient. *In situ* it shows tonic pulse waves running with a higher frequency (F:30-50/') and being under a higher pressure (Sys./Dias. : 294-390/98-196 Pa) than the peristaltic pulse waves of *Nautilus* (Gosline and Shadwick 1982; Schipp and Kleemann 1994).

This study aims at presenting some further cytological and histochemical aspects of the cephalic aorta of *Sepia officinalis*. They could contribute to a more comprehensive understanding of the tonic functions and the neuronal control of this vessel type (Schipp *et al.* 1991; Schipp and Fiedler 1994).

MATERIALS AND METHODS

Juvenile *Sepia officinalis* L. (mantle length : 3-6 cm) from the Bassin d'Arcachon (Atlantic Ocean) and adult animals (mantle length : 9-10 cm) from the Mediterranean near Banyuls-sur-Mer were used in this study. All animals were anaesthetised by 1.5 % ethanol/seawater before dissections were carried out.

LM-Methods : For the histological analyses vessel preparations fixed in Bouin's solution or 3.5 % glutar-

aldehyde PBS were embedded in paraffin or araldite. Araldite sections (1 µm) were studied in the phase-contrast microscope. Paraffin sections (6 µm) were stained with the Masson differential coloration or the nerve silver impregnation after Bodian.

TEM-Methods : Tissue specimens fixed with 4 % glutaraldehyde and 1.5 % OsO_4 (PBS; pH 7.3; 1100 mOsm) were embedded in araldite and the ultra-thin sections viewed in a Philips EM 300.

The freeze-etching preparations were made with a modified method after Bachmann *et al.* (1969). The fixed specimens, frozen in liquid nitrogen (– 196 °C) were cleaved at – 100 °C under 10^{-6}-10^{-7} Torr and rotatory-shadowed with platinum and carbon in a Balzers Mikro-BA3 at an angle of 45°. Replicas were floated in a detergent including NaClO and after rinsing in A. dest. mounted on uncoated copper grids.

Histochemical methods : specific staining of elastic fibres was obtained by aldehyde fuchsin (Gomori 1950) and the PAS-reaction demonstration of catecholaminergic nerves by glyoxyl acid induced fluorescences (de la Torre and Surgeon, 1976, modified by Barber, 1982); localization of the acetylcholinesterase (AChE, E.C. No. : 3.1.1.7) and the non-specific cholinesterase (EC. No : 3.1.1.6) was made after Karnowsky and Roots (1964) using ISO-OMPA as inhibitor of the non-specific Ch-E. Immunhistochemical and cytochemical localization of FMRFamide and serotonine (5-HT) were made using the peroxidase-anti-peroxidase (PAP) reaction and colloidal gold (∅ 15 nm) as tracer (Sternberger *et al.* 1970).

RESULTS

Morphological findings

The wall of the cephalic aorta of *Sepia officinalis* L. consists of 3 layers (Plate I A) : 1. Tunica intima (T.i.). It is composed of an incomplete endothelium and a PAS-positive lamina basalis which in semi-adult and adult animals is lined with an elastic layer that shows a positive reaction to aldehyde fuchsin. 2. Tunica media (T.m.). Depending on the age of the animals it is built by a varying number of circular muscle layers which are surrounded by a network of collagenous fibres. In adult and semi-adult animals elastic fibres take also part in the intercellular matrix. Most of them are circularly arranged, but there are also singular radially running fibres connecting the T. intima with the T. media and the peripheral wall area. 3. Tunica adventitia (T.a.). Its inner part is built by longitudinal and transversal muscle fibres that are peripherally lined by a different number of layers of fibrocytes and circular and transversal collagenous fibres. In addition to numerous afferent and efferent (= exchange) vessels (vasa vasorum), there are, locally, close formations of polyaxonal nerve fibres. Their terminal endings enter into the peripheral T.m., but do not occur within its middle and inner part.

Cytological findings

According to TEM analyses the *elastic fibres* which are typical for the T.i. and T.m. of older animals, are in close contact with the sarcolemma of bordering muscle cells. They are built up by bundles of fibrils that are connected by an amorphous electron dense material. The fibrils have nearly the same diameter (11-16 nm) as those of the adjacent collagenous fibres but do not show their typical periodic cross striation (Plate I C, D).

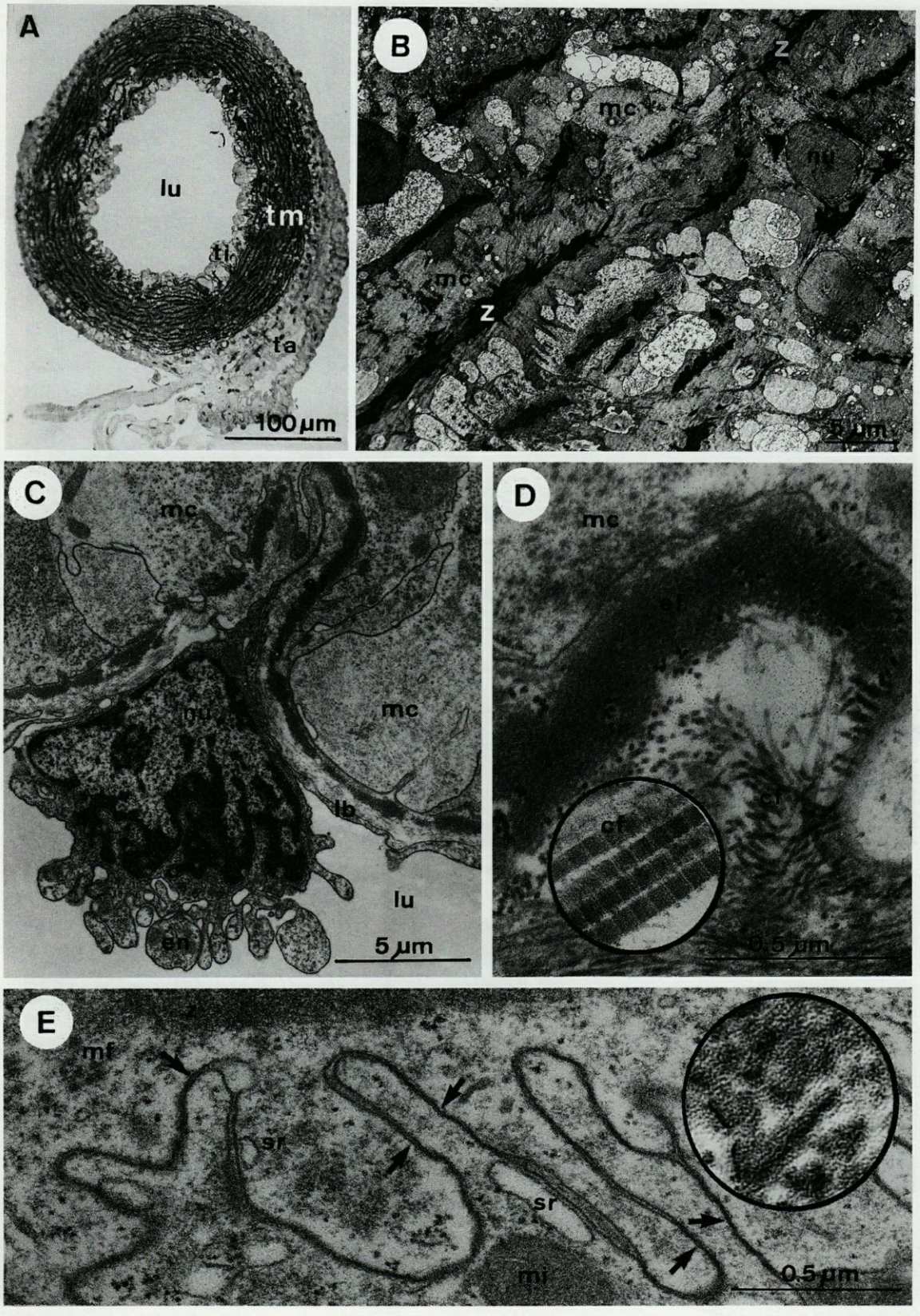
The *muscle cells* have only a small number of sarcosomes. They are closely interdigitated; their extended areas of gap junctions which show the typical pattern of hexagonal connexions in freeze-etching TEM-preparations are one of their main characteristics (Plate I E). The extended velum-like Z-patches anchored by 10 nm filaments within the cytoskeleton and in hemidesmosomes of the sarcolemma can be seen as a further special feature of these muscle cells (Plate I B, II A). The T-system is represented by funnel-shaped tubules deeply entering the muscle cells at the level of the Z-patches. Numerous close membrane contacts between SR-tubules and the sarcolemma occur especially in the area of gap junctions (Plate I E).

The neuro-muscular junctions

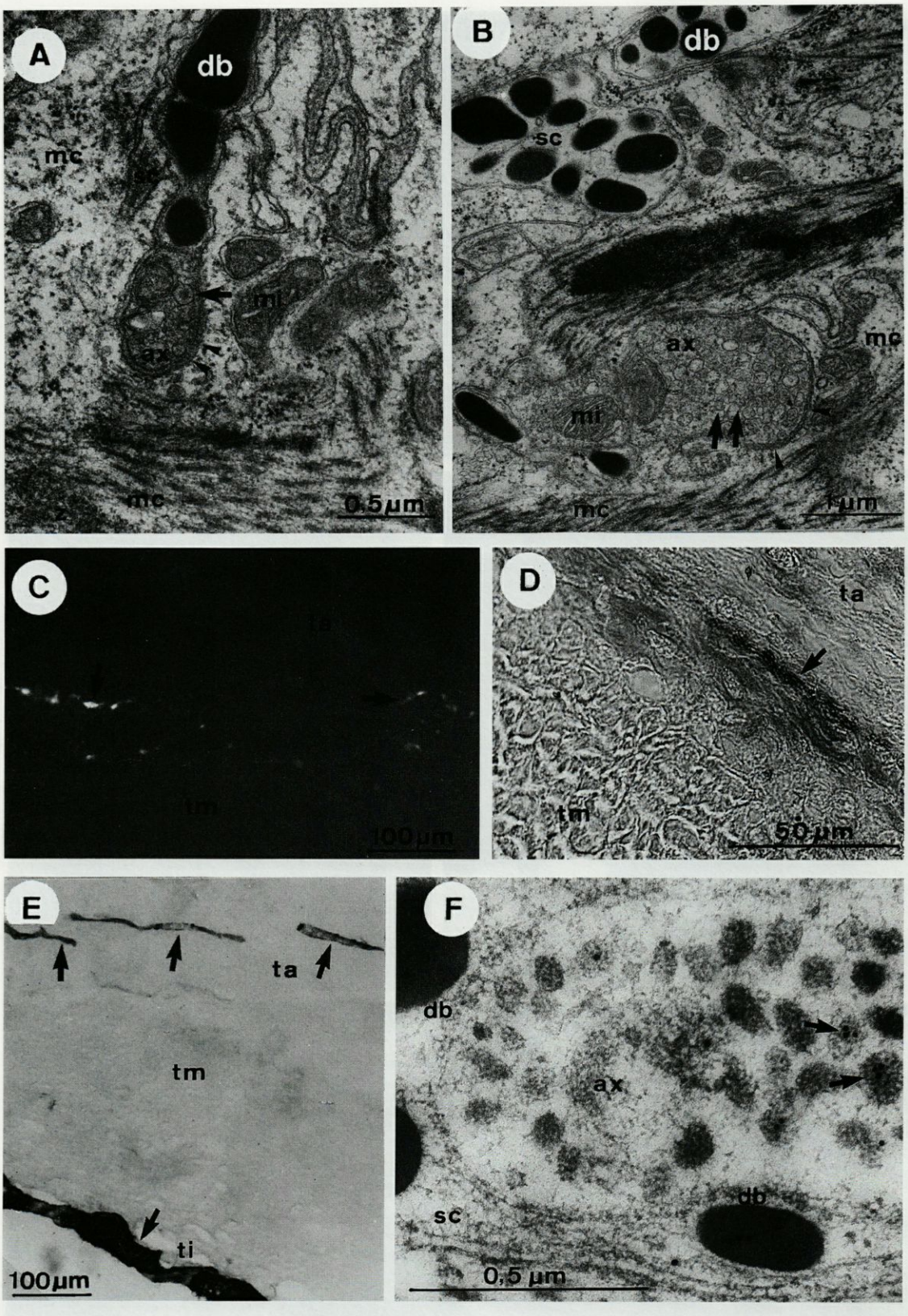
The terminal nerves reach only the peripheral muscle cells of the T.m. In contrast to the polyaxonal nerve fibres of the T.a. they contain few axons only which are surrounded by a sheath of Schwann cells on one side only. In the synaptical area the non-sheathed sides of the axons are in close and extensive contact with the post-synaptic membrane (= sarcolemma) and often seem to be deepened into crypt-like sarcolemmal invaginations comparable to the neuro-muscular junctions of the motor endplate. The intersynaptic cleft has a width of 15-20 nm. The axons contain different types of vesicles : large transparent vesicles (∅ 138 ± 25 nm), dense cored vesicles (∅ 102 ± 23 nm), small dense bodies (∅ 170 ± 45 nm) and accumulations of small transparent synaptical vesicles at the presynaptic membrane (∅ 62 ± 11 nm). Large dense bodies (± 540 ± 365 nm) are typical for the Schwann cells (Plate II A, B).

Histochemical results

The ACh-E- as well as the Ch-E-reactions revealed strong Hatchet-Brown colorations within the whole area of the T.i. (= lamina basalis) and distinct spot- or fibre-like pigmentation at the border of the T.a. and the T.m., i.e. the area of the neuro-muscular junctions; but the non-specific



Pl. I. – Cephalic aorta of a juvenile *Sepia officinalis*. A, Total cross section alcian blue stained (in LM). B, Cross section from the T. media in TEM with circular muscle cells that are closely interdigitating. C, The area of the T. intima shows elastic fibres in the stage of forming in close contact with muscle cells. D, partial TEM section from C at higher magnification (inset magnification : 69900X). E, Close interdigitations of muscle cells within the T.m. with numerous gap junctions (arrows) in close contact with sr-tubuli in TEM and a freeze-etching preparation (inset magnification : 110000X). ax, axon; cf, collagenous fibres; db, dense bodies; ef, elastic fibres; en, endothelium; lb, lamina basalis; lu, lumen; mc, muscle cell; mi, mitochondrium; nu, nucleus; sc, Schwann cell; sr, sarcoplasmic reticulum; ta, Tunica adventitia; ti, Tunica intima; tm, Tunica media; z, z-patches.



Pl. II. – Innervation of the cephalic aorta of *Sepia officinalis*. A, B, Neuromuscular synapses with dense cored vesicle (arrow) and transparent vesicles of different sizes (double arrow), synaptic area (arrow heads). C, Glyoxyl acid induced fluorescences in the border area of t.a./t.m. (arrows). D, Immunhistochemical reaction against FMRFamide (arrow) within terminal nerves of border area t.a./t.m. E, Acetylcholinesterase-reaction of the nerve endings of the t.a. and the t.m. F, Immunogold reaction against FMRFamide within electron dense granules (arrows) and dense bodies of a Schwann cell. Abbreviations as in Plate I.

Ch-E-reaction was stronger at the T.i. than at the area of the neuro-muscular junctions where a stronger reaction of the ACh-E was obvious (Plate II E).

Glyoxyl acid-induced fluorescences (E max : 480 nm) were localized only within the area of the neuro-muscular junctions at the border T.a./T.m. These were distinct but not frequent and showed a fibre- or bead string-like pattern (Plate II C).

The immunhistochemical methods applied produced positive reactions of the polyclonal-A.B. against FMRFamide but not of that against 5-HT. FMRFamide positive PAP reactions with spot- or fibre-like colorations occurred within the area of the neuro-muscular junctions at the border of T.a./T.m., and in larger nerve fibres within the peripheral T.a., singular spot-like PAP-colorations could also be detected within the T.m. (Plate II D). Small dense bodies within terminal axons and larger ones within the Schwann cells were traced by the immunogold-reaction against this peptide (Plate II F).

DISCUSSION

The structure of the T.i. of the cephalic aorta of adult *Sepia officinalis* resembles that of the adult *Octopus* aorta (Barber and Graziadei 1967; Shadwick and Gosline 1983). In both instances there is an incomplete endothelial layer but a continuous lamina basalis which is built by a collagenous network and a layer of elastic fibres.

As to our findings showing that in juvenile animals elastic fibres are not yet established, comparable results from octopods do not exist. The close contact of the elastic fibres, especially of their initial cores of formation in juvenile animals, to the sarcolemma as well as their substructure and diameter of fibrils suggest that the elastomere protein and/or its precursor substances are synthetized and secreted by the muscle cells. Furthermore it is probable that these precursor substances penetrate into an already preformed collagenous network. By the increasing cross linking of its fibrils the latter seem to lose their typical cross striation and to get a secondary amorphous substructure of high electron density.

The second structural peculiarity that is responsible for the special elastic/tonic properties of this vessel type, in sepioids as in octopods, is the highly specialised muscle system of the T.m. with its irregular cross striation. The extended velum-like Z-patches look similar in sepioids and octopods. They can be interpreted as a special structural adaptation of this high pressure vessel since they are not established in the nautiloid aorta that is under a distinct, lower pressure (Bourne *et al.* 1978; Shadwick and Gosline 1983, 1985a, b; Schipp and Kleemann 1994).

Our TEM analysis revealed, in the area of the close interdigitations between the muscle cells, extended gap junctions with the typical connexon-pattern which are in a close topical relationship to SR-tubules and their marginal cisterns. These structures suggest an electrical coupling of the T. media muscle system, thus representing a functional syncytium.

Our LM- and TEM findings indicate that only the peripheral muscle cells of the T.m. are reached by nerve endings forming close neuro-muscular junctions comparable to the axon-muscle couplings of the motor endplate; this means that these peripheral muscle cells probably have a pace-maker function influencing the cells of the inner T.m. via the gap junctions.

The histochemial results about the ACh-E activity, and the catecholamines indicating fluorescence within terminal nerves of this area correspond to the cytological finding that transparent as well as small dense cored neuro-vesicles were detected within the terminal axons. These structural evidences for a dual cholinergic-catecholaminergic innervation of the cephalic aorta of *Sepia* are in accordance with findings on other cephalopod vessels (Andrews and Tansey 1983; Schipp 1977; Kleemann and Schipp 1996) as well as earlier physio-pharmacological results on the *Sepia* aorta (Schipp *et al.* 1991; Schipp and Fiedler 1994).

The positive immunhisto- and cytochemical reactions against FMRFamide can probably be related to medium-sized dense bodies within the terminal axons localized by TEM. They are also in accordance with functional findings about vasodilatatory actions of this peptide on isolated aorta preparations precontracted by dopamine (Schipp *et al.* 1991).

The role of 5-HT in the neuroregulation of this vessel remains to be clarified. In pharmacological tests it showed also a vessel tonus decreasing effect, but the histo– and immunhistochemical method applied in this work did not reveal this neurotransmitter; a possible humoral action of this and other neurotransmitters via the bloodstream has to be taken into consideration.

The function of the high ACh-/BUT-Ch-E-activity (EC No : 3.1.1.7/3.1.1.8) within the lamina basalis of the T.i. is not yet clarified either; the findings confirm similar strong reactions of these enzymes in other coleoid and nautiloid vessels and heart organs and suggest that the lamina basalis represents an extended ACh-/Ch-barrier of the circulatory system of cephalopods, possibly protecting the inner wall area against cholinesters circulating in the blood (Schipp 1977, 1987; Kling 1986; Kleemann and Schipp 1996).

ACKNOWLEDGEMENTS. – This study is dedicated to Prof. R. Baessler, Fulda, in honour of his 70th birthday. The authors thank Prof. A. Guille and Dr. S. v. Boletzky, Laboratoire Arago, Banyuls; Prof. C. Cazaux and Prof. M. Caumette, Laboratoire d'Océanographie Biologique, Arcachon, who provided the facilities for work in their laboratories; H. Côté, A. Hudel, Institute of Zoology, and G. Magdowski, Institute of Anatomy and Cytobiology, Justus-Liebig-Universität Giessen, for their valuable technical assistance. The investigations were supported by grants of the "Deutsche Forschungsgemeinschaft".

REFERENCES

ANDREWS P.L.R. and TANSEY E.M. 1983. Aminergic innervation of the blood vessels of *Octopus vulgaris*. *Cell Tissue Res.* **230** : 229-232.

BACHMANN L., ABERMANN R. and ZINGSHEIM H.P. 1969. Hochauflösende Gefrierätzung. *Histochemie* **20** : 133-142.

BARBER A. 1982. Monoamine-containing varicosities in the neural sheath of gastropod mollusc demonstrated by a glyoxylic acid histofluorescence. *Cell Tissue Res.* **226** : 267-273.

BARBER V.C. and GRAZIADEI P. 1967. The fine structure of cephalopod blood vessels III. Vessel innervation. *Z. Zellforsch.* **77** : 162-174.

BOURNE G.B. 1982. Blood pressure in the squid, *Loligo pealei*. *Comp. Biochem. Physiol.* A **72** : 23-27.

BOURNE G.B., REDMOND J.R. and JOHANSEN K. 1978. Some aspects of hemodynamics in *Nautilus pompilius*. *J. exp. Zool.* **205** : 63-70.

GOMORI G. 1950. Aldehyde Fuchsin : a new stain for elastic tissues. *Am. clin. Path.* **20** : 665-666.

GOSLINE J.M. and SHADWICK R.E. 1982. The biomechanics of the arteries of *Nautilus*, *Nototodarus*, and *Sepia*. *Pacific Science* **36** : 283-296.

KARNOWSKY M.J. and ROOTS L. 1964. A "direct-coloring" thiocholin method for cholinesterase. *J. Histochem. Cytochem.* **12** : 219-221.

KLEEMANN S. and SCHIPP R. 1997. Some histological and cytological aspects of smaller arteries of *Nautilus pompilius* (L.) and *Nautilus macromphalus* (G.B. Sowerby). *Vie Milieu* 47 (2) : 117-121.

KLING G. 1986. Histochemical localization of cholinesterases and monoamines in the central heart of *Sepia officinalis* L. (Ceph.). *Histochemistry* **85** : 241-250.

SCHIPP R. 1977. An indirect demonstration of the substructure of the lamina basalis in the branchial heart of *Sepia officinalis* L. by means of cholinesterase reaction. *Experientia* **33** : 74-76.

SCHIPP R. 1987. The blood vessels of cephalopods. A comparative morphological and functional survey. *Experientia* **43** : 525-537.

SCHIPP R. and FIEDLER A. 1994. Cholinergic mechanisms in the neurocontrol of the cephalic aorta of the cephalopod *Sepia officinalis*. *Comp. Biochem. Physiol. C* **107** : 149-157.

SCHIPP R. and KLEEMANN S. 1994. Vergleichende Untersuchungen zur Funktionsmorphologie der Aorta cephalica von Nautiliden und Coleoiden (Cephalopoda). *Verhandl. Dt. Zool. Ges.* **87** (1) : 111.

SCHIPP R., JAKOBS P.M. and FIEDLER A. 1991. Monoaminergic-peptidergic interactions in neuroregulatory control of the cephalic aorta in *Sepia officinalis* L. (Cephalopoda). *Comp. Biochem. Physiol. C.* **99** : 421-429.

SHADWICK R.E. and GOSLINE J.M. 1983. The structural organisation of an elastic fibre network in the aorta of the cephalopod *Octopus dofleini*. *Can. J. Zool.* **61** : 1866-1879.

SHADWICK R.E. and GOSLINE J.M. 1985a. Physical and chemical properties of rubber-like elastic fibres from the *Octopus* aorta. *J. Exp. Biol.* **114** : 239-257.

SHADWICK R.E. and GOSLINE J.M. 1985b. Mechanical properties of the *Octopus* aorta. *J. Exp. Biol.* **114** : 259-284.

STERNBERGER L.A., HARDY P.H., CUCULIS J.J. and MEYER H.G. 1970. The unlabeled antibody enzyme method of immunohistochemistry- preparation and properties of soluble antigen-antibody complex (Horseradish peroxidase-Antihorseradish Peroxidase) and its use in identification of Spirocetes. *J. Histochem. Cytochem.* **18**(5) : 315-333.

TORRE de la J.C. and SURGEON J.W. 1976. A methodical approach to rapid and sensitive monoamine histofluorescence using a modified glyoxylic acid technique : *The SPG method. Histochemistry* **49** : 81-93.

Reçu le 4 avril 1996; received April 4, 1996
Accepté le 16 octobre 1996; accepted October 16, 1996