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0014-4754/87/050511-15$1.50 + 0.20/0
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The blood vessels of cephalopods. A comparative morphological and functional survey

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Summary. The distinctive structural and functional features of the highly specialized blood vessel types (blood sinus, veins, aortae, arteries) of cephalopods are characterized in a comparative survey. Histochemical and physio-pharmacological results indicate a dual neuroregulation of the vessel wall but, however, show contrary effects of the neurotransmitter candidates, catecholamines, serotonin (5-HT) and ACh, on arteries and veins. The spontaneous pulsatile activity of the propulsive veins seems to be partly myogenic but also directly dependent on a dense cholinergic innervation of the sarcosome-rich obliquely striated myocytes.
Key words. Cephalopods; blood vessel; wall structure; innervation; neurotransmitter effects.

Introduction

In the introductory paper of this multi-author review reference was already made to the connection between the particular evolutionary stage of the cephalopods and the differentiation and efficiency of their circulatory system. Therefore it is clear that the specialized vessel system, found in no other invertebrate organism, should become the subject matter of topographical-anatomical systematists and that the numerous arteries, veins and sinus of the different groups of cephalopods should be accorded a very exact classification and nomenclature (*Nautilus*[26, 73]; *Eledone*[28]; *Sepia*[65]; *Loligo*[74]). There are, however, surprisingly few investigations focussing on their structure and physiology. Only more recent cytological and physiopharmacological results help to throw light on their functional peculiarities, i.e. points of similarities and differences when compared to the vessels of vertebrates.

Blood pathways, transporting forces and functional vessel types

In both nautiloids and coleoids there is a highly differentiated system of arteries (see fig. 1 and 2 in the introductory paper to this multi-author review). It begins distal of the ventriculo-aortic semilunar valve of the systemic heart with large resistance vessels, aorta cephalica, aorta abdominalis. These, along with the adjoining peripheric resistance vessels – smaller arteries, arterioles – act as a 'Windkessel' owing to the elasticity of their walls (fig. 1). They transfer the pulsating blood flow, close to the heart, with relatively high pressure values (table 1 in the introductory paper) towards the periphery in a continuous laminary flow with lower pressure. Only in *Nautilus* could an autorhythmic contractility of the aorta cephalica be observed as an additional transporting force[14] (personal observations, 1982).

The exchange vessel system within the peripheric organs – brain, locomotory muscles, digestive and genital tract, sense apparatus etc. – is exclusively an extensive blood sinus[26] in nautiloids, whereas in coleoids there is a far stronger microvasculature[15, 59] ('true capillaries'[31]), that can reach a density of 45 vessels per mm^2 (lower vertebrates 300 mm^2)[16, 17]. But also in coleoids endothelial-less sinuses are developed especially in the region of the head, but also in the digestive and renal tracts. These tissues, like the more or less hemocyanin free extravasal 'tissue channels'[18] or 'lymphoid channels' in the CNS[62], have a distributive function and they, together with the collecting veins and bulbi form the capacity vessel system[31, 70]. In both systematic groups the back flow of the venous blood is effected through large propulsive veins (e.g. pharyngo-ophthalmic and posterior mantle vein, *V. cephalica*, efferent branchial vessel)[14, 21, 23, 45, 48, 58], supported by forces generated by the muscles of body walls[31], the autonomous contractility of muscle network within the sinus of the peripheric organs (e.g. gills, renal and pericardial appendages, midgut gland of *Nautilus* and in coleoids also by the branchial heart contractility (see contribution Fiedler/Schipp in this review)).

The general vessel architecture

'The large vessels' of cephalopods, irrespective of caliber and vessel type, have a more or less 3-layered wall structure, like that in vertebrates[32,35]. The T. intima consists of an endothelium which is often incomplete, and the large lamina basalis which is always continuous. It is the most important barrier between the hemocyanin-containing blood and the intercellular space of tissue channels of the supplied organs[18]. The pericytes, which are often closely adjacent to the lamina basalis, are often regarded as belonging to the T. intima, whereas the T. media is usually composed of a loose connective tissue and circular and longitudinally arranged fibers of obliquely striated muscle cells. The composition of the T. adventitia is a collagenous network with a large number of nerve fibers. It keeps the vessel firmly positioned in the surrounding tissue and, in the case of larger vessels, also contains vasa vasorum or a marginal sinus[8-11,32,39,68].

Cyto-functional aspects of the efferent vessels

Among the arteries it is the aorta (Ao. cephalica, Ao. abdominalis) which show some cytological particularities[11,39], adapting them to the very high blood pressure found there and to the above-mentioned 'Windkessel' function[14,31,69] (figs 2 and 3). The branched interdigitated endothelial cells of the T. intima form a more or less tight layer; their intercellular gaps are, however, not tight, but have variable spaces (200 Å – 2–3 µm), so that this layer is reminiscent of podocytes in renal organs. Striking features are the ovoid, PAS-positive dense bodies that are obviously discharged to the wall side by exocytosis. There they are incorporated initially as patch-shaped distributed matrix substance, into the very fine collagenous network of the large lamina basalis (∅ 0.5–3 µm)[39]. The T. media consists of several circular interdigitated muscle layers (*Sepia officinalis, Eledone cirrhosa:* 6–8 in juvenile and 15 or more in adult animals) (figs 2 and 3). A loose 3–4 layered longitudinal fiber area adjoins this and its extending fibers branch off peripherally into the T. adventitia.

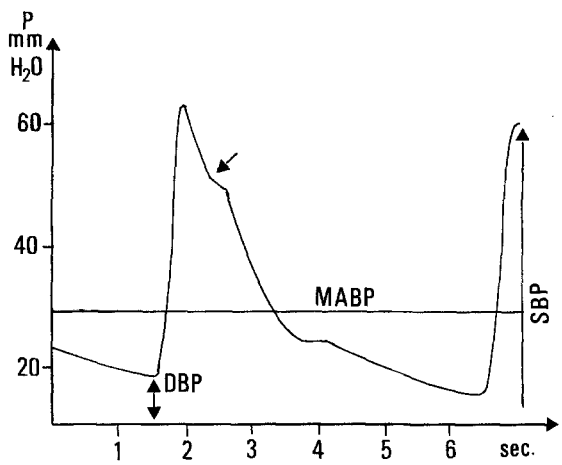
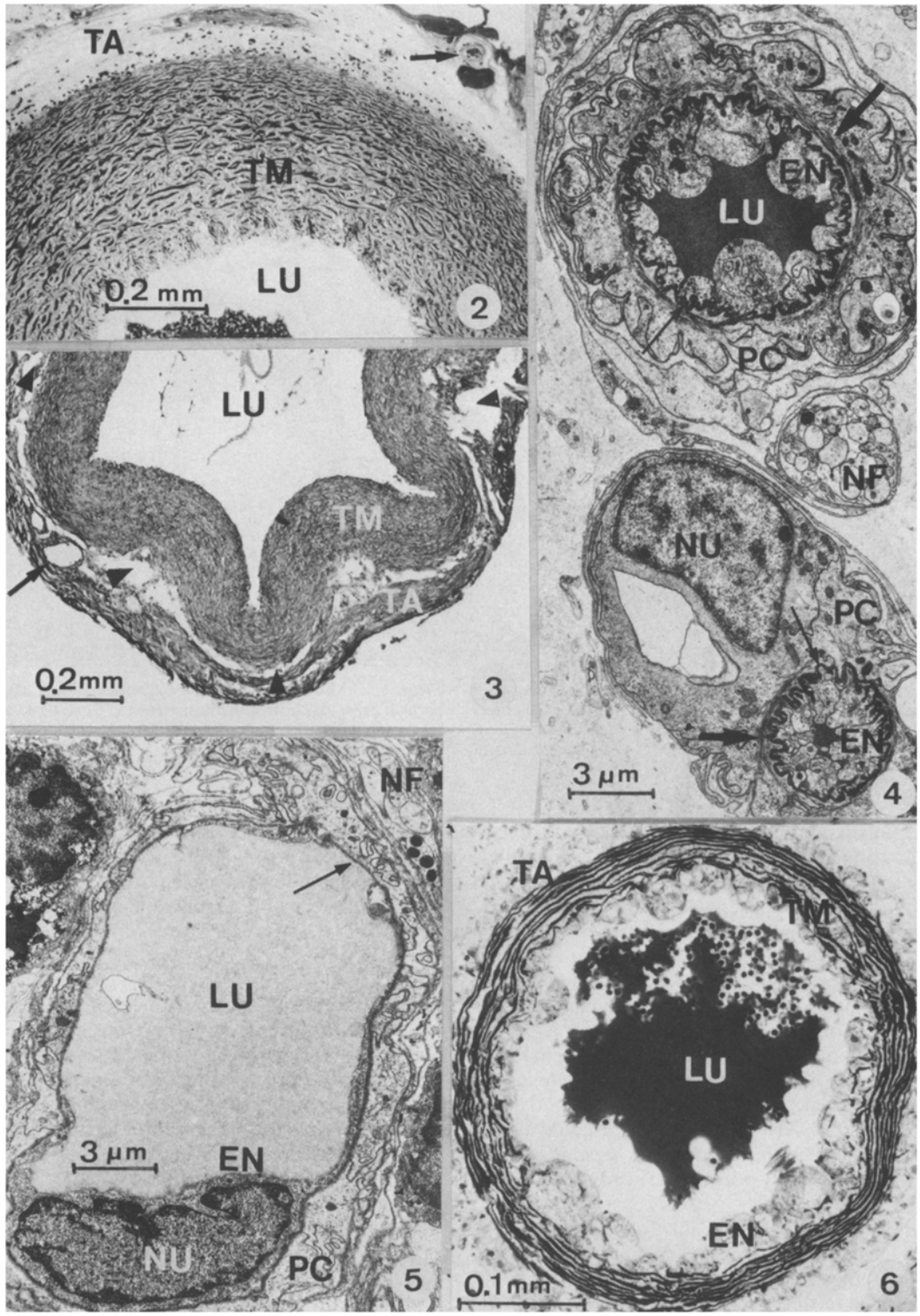


Figure 1. Aortic pulse wave of *Sepia officinalis* with the typical dicrotic notch (Incisura, I) caused by the retrograde surge of blood closing the semilunar aortic valves; mean aortic blood pressure (MABP), diastolic and systolic blood pressure (DBP/SBP).

According to our results on different coleoids these mononuclear muscle cells represent an independent type that differs quite distinctly from the obliquely striated muscles of the smaller arteries, veins and heart organs[51,52]. A noticeable feature is *one* central z-patch rope per cell that branches laterally in 3 dimensions, thus contacting the sarcolemma (fig. 7). The multi-directional oriented acto-myosin filament system is inserted between these many z-patch processes – which are probably composed of α-actinin – and to a lesser extent at the sarcolemma. The sarcoplasm is limited to the cell periphery with only a few filaments, where voluminous cell-bulges between the z-patch contact areas contain only few sarcosomes, irregular sarcotubuli, vacuoles and the nucleus. Processes from neighboring cells appear in funnel-shaped invaginations on the level of the z-patch contact areas – they are to be taken functionally as t-system. Thus a tightly closing but also very elastic muscle-structure is formed, that can be regarded as a structural correlate for the already-mentioned elastic tonic qualities of the coleoid aorta. The T. adventitia mainly contains ER-rich fibroblasts that produce an extracellular matrix which appears to be homogeneous when examined using the TEM, as well as collagenous fibers. This matrix, along with the above-mentioned endothelial dense bodies within the lamina basalis, possibly represents the 'arterial elastomer proteins' isolated by Shadwick and Gosline[57] out of the *Octopus* aorta. Furthermore, polyaxonal nerve fibers are found in this layer which only contact peripheral muscle cells and hardly seem to penetrate into the T. media, and vasa vasorum (= small arteries and collecting veins) are also found (figs 4 and 5).

The latter – according to our findings in nautiloid aortae – obviously seem to be replaced there by a collecting marginal sinus that seems to have a link to the large body sinus and the V. cava system (fig. 3).

Cytological results on medium sized and small arteries (fig. 6) are few and far between. They refer to 'smaller vessels' of the periesophageal ganglion of *Octopus vulgaris*, the stellate ganglion of *Sepia officinalis*[10], brachial arteries of *Octopus*[59], *Sepia Sepiola*[7], and the cutaneous layer of the mantle of *Sepia esculenta*[35]. The authors mentioned usually give a classification of vessels according to structure but often not clearly referring to arteries or veins. A more exact investigation concerning the branchial artery of *Eledone cirrhosa* (∅ 1 mm) has been made[39]. The T. intima of this vessel resembles that of the coleoid aorta cephalica, whereby the above-mentioned PAS-positive endothelial dense bodies seem also to participate in the formation of a 2–3-µm thick lamina basalis. On the other side of the lamina basalis, still counting as part of the T. intima, pericytes containing myofilaments join on. The T. media consists of an inner collagenous fiber layer with ER-rich fibrocytes, followed by 2–3 interdigitated layers of small circular muscle cells (∅ 15–25 µm); these are of the obliquely striated type, thus corresponding principally to the myocard cells in their fine structure[51,52], and also to the body muscles – more closely examined in coleoid tentacles[24] or in the digestive tract of Sepia[5]. The sarcosome content is higher than that of the aorta muscle cells. They also have a t-system, though not a well-defined one in view of their small diameter, and it contacts the intercellular sarcotubuli via diades.



Figures 2 and 3. LM sections of the aorta cephalica of a semiadult *Sepia officinalis* and an adult *Nautilus macromphalus;* multilayered T. media (TM), T. adventitia (TA), lumen (LU), afferent vasa vasorum (arrow), collecting marginal sinus (arrow heads).

Figures 4 and 5. TEM sections of afferent and efferent vasa vasorum from the T. adventitia of the cephalic aorta of *Sepia officinalis:* endothelium (EN), lamina basalis (small arrows); note the different content of myofilaments within the pericytes (PC) of two vessel types (big arrows); nucleus (NU), polyaxonal nerve fibers (NF); further abbreviations as in figs 2 and 3.

Figure 6. LM-section of the afferent branchial vessel (= branchial artery) of *Loligo vulgaris* with very distinctly marked circular muscle fibers within the T. media; blood cells (BC); further abbreviations as in figs 2 and 3.

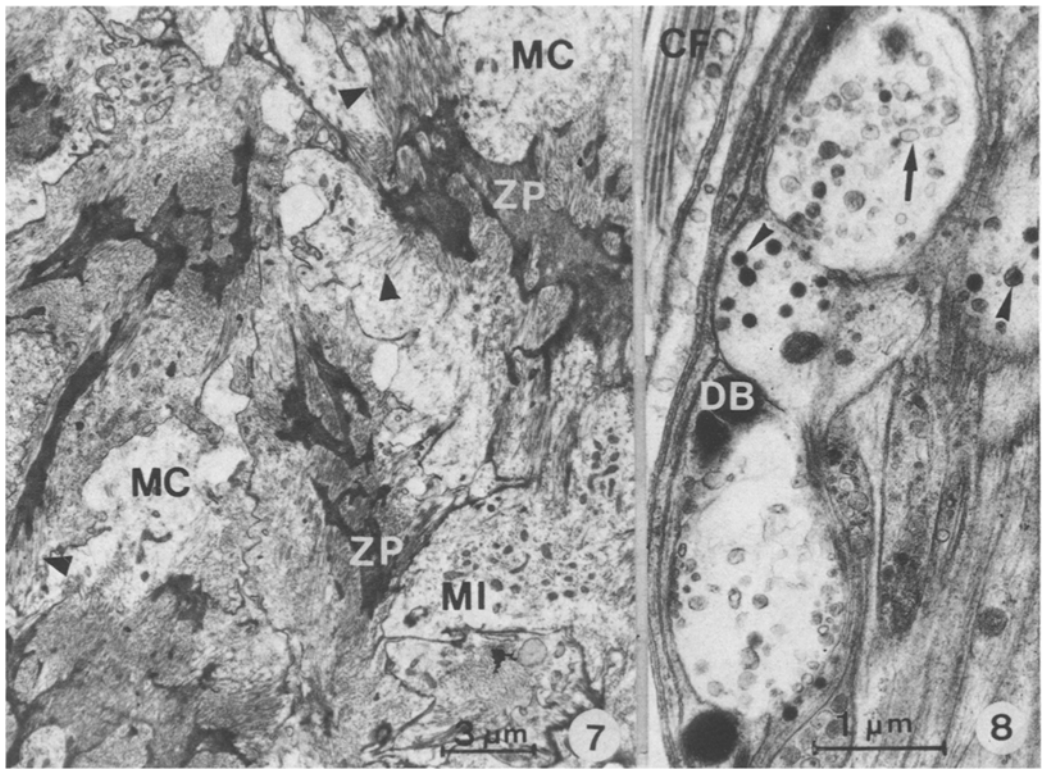


Figure 7. Cephalic aorta of a semi-adult *Sepia officinalis;* TEM-section of the inner part of the T. media; note the extended branched z-patch ropes (ZP) within the central areas of the closely interdigitated muscle cells (MC) and the few content of mitochondria (MI); myofilaments (arrow heads).

Figure 8. TEM of a polyaxonal nerve fiber from the T. adventitia of the aorta of *Sepia officinalis* with transparent and dense cored vesicles within the axons (arrow/arrow heads) and dense bodies within the glia cells (DB); collagenous fibers (CF).

Numerous polyaxonal nerve fibers are brought forward through the collagenous T. adventitia, thus every muscle cell seems to be reached by nerve endings. Elastic elements have not yet been observed.

Barber and Graziadei[8] classify the 'smaller vessels' in retina and lip of *Octopus vulgaris* and *Sepia officinalis* into two main types. Type 1 vessels, 'considered to be equivalent to small arterioles' (p. 766) are characterized by a complete lamina basalis – folded in contracted vessels – and an 'incomplete lining of endothelial cells formed into finger-like processes and a complete investment of myofilament containing pericytes tightly surrounding the vessels'. Arterioles of this type could also be found as vasa vasorum in the aortae of *Sepia officinalis,* and are also innervated by polyaxonal nerve fibers there[11] (fig. 4).

The vessels of type 2 which 'probably follow on from type 1 vessels in the circulatory system'[8] (p. 771) we could also find both among the vasa vasorum of the aorta and within larger nerve fibers in the auricles of *Sepia officinalis* (fig. 5). These somewhat smaller vessels, varying in caliber and diameter of lumen, reveal a flat endothelium which may even be absent over large areas, also a much thinner lamina basalis and pericyte-cover with only a few myofilaments; they are, however, accompanied by polyaxonal nerve fibers. The 'exchange vessels' or 'capillaries' (∅ lumen: 15 µm, wall: 0.25 µm) described in the brachial muscle of *Octopus pallidus*[16,17] are included with them, as are the exchange vessels in the brain of *Octopus vulgaris*[25], termed capillaries; myofilament content and nerve supply of the pericytes indicate that these exchange vessels, like those of type 1, can also regulate the pressure and peripheric flows of the blood by a tonic contractility which is powered by a neural control mechanism[35].

The vessel permeability

Tracer experiments on the permeability of the wall of the mentioned 'exchange vessels' revealed that particles of ferritin (∅ 110 Å), but not of carbon, can penetrate the lamina basalis directly with a certain retardation, whereas in the adjacent pericytes 'the junctions appeared to be the main avenue for movement of ferritin'[16]. Gray[25] finds single instances of hemocyanin molecules in tissue channels of the *Octopus* brain. In accordance with this, the lamina basalis of a blood sinus does not constitute an absolute 'blood barrier' in the hemocyanin-synthesizing organs; branchial gland in coleoids[44,53], midgut gland in *Nautilus*[49,55]. That means that it can be passed locally under special physiological conditions by these macromolecules. The passage of neuropeptide particles into the lumen of the veins, which is yet to be discussed, functions on a similar principle (figs 16 and 17).

The cyto-morphological aspects of the veins

The histological and cytological results on collecting and transporting veins of medium-sized and larger calibers are not very numerous either. Yet the V. cephalica of coleoids[3,4,13,42,68], and the similarly autonomous contrac-

tile brachial vein of *Octopus dofleini*[59], have been well examined, as have the efferent branchial vein in *Sepia officinalis*[68] and in *Nautilus*[49,50]. Despite certain caliber-dependent variations in the quantity and distribution of different structural elements, the mentioned veins have – with the exception of the extremely muscular and trabecular V. cephalica of *Nautilus* (figs 20 and 21) – a relatively unified basic type of wall structure. The T. intima resembles the larger arteries. Here too there is a more or less tight endothelial layer with variable intercellular gaps, whose cells discharge rather large dense bodies abluminally (fig. 13) to form the 0.2–0.3 µm thick lamina basalis[68]. A loose collagenous network with several longitudinal-transversally arranged muscle cells, different in number in the various veins, joins on the irregularly folded lamina basalis. This network continues (without a clear border) in the T. media, where there are only 2–4 circular layers of obliquely striated muscle cells pass (fig. 9) – considerably fewer than in arteries of a comparable size. The muscle cells, corresponding to their pulsative contractility, are particularly rich in sarcosomes (fig. 11) and, with their different diameter, they have sometimes only a suggestion of a t-system, but also deep tubular invaginations (figs 9 and 12). Apart from a large number of vasa vasorum the high density of nerve fibers in the T. adventitia and T. media (fig. 21) should be pointed out, suggesting that every muscle cell is reached by nerve endings. The terminal axons contain both dense cored and transparent vesicles (∅ 70–120 nm), neurotubuli and single mitochondria; as in the glia cells of the nerve terminals of heart organs, numerous dense bodies (∅ 0.1-1 µm) can also be found (fig. 10).

The fine structure of the neuromuscular synapsis in the vessel wall is generally characterized by the typical accumulation of small transparent synaptic vesicles (∅ 50–60 nm) on the presynaptic membrane (fig. 10). The intersynaptic cleft has a diameter of 20 nm, but in the case of pulsative veins it must be assumed that a larger and variable cleft exists between axon and muscle and there is a 'synapse par distance'[29] in many cases.

It is worth mentioning that in some of the vessels examined, like the V. cephalica, V. ophthalmica of coleoids (the so-called NSV-system[3,4]) or efferent branchial vessels of *Nautilus* (fig. 19), terminal enlarged axons penetrate the T. media and intima, luminally limited only by a loosened lamina basalis, to border directly on the vessel lumen, where neurosecretions are obviously released by exocytosis (figs 16 and 17), a functional-morphological aspect to be dealt with in the following chapter. Furthermore it is worth noting that many free nerve fibers surrounded by hemolymph were found, also within the blood sinus of *Nautilus*. They innervate there the spongy meshwork of obliquely striated muscle cells.

With regard to the above-mentioned significance of the vis a tergo for the blood flow in the veins[31], the frequently-appearing muscle fibers of the periadventitial area deserve a reference. Very strong longitudinal muscles run between the vessel wall in sensu stricto of V. cephalica and the mantle cavity epithelium (fig. 14). These muscles might be important as a mechanical protection against over-stretching considering the changing pressures of the respiratory water flow; also due to their autonomous contractility for blood flow, which has yet to be discussed in more detail.

In addition it should be said that iridocytes have also been demonstrated within the periadventitial area (efferent branchial vessels of *Sepia officinalis*); their leucosome

Table 1. Effects of neurotransmitters on the aorta cephalica of coleoids

Species	Applied substances	Method of application	Applied doses	Responses	References
Octopus dofleini	Noradrenaline	in situ	50–100 µg	Depressing; decrease of the peripheral arterial vascular bed	Johansen and Huston[30]
	adrenaline	in situ	50–100 µg		
	Tyramine	in situ	100 µg	Catecholamine mimese	
	Serotonin (5-HT)	in situ	100 µg	Increase of systolic pulse pressure, no diastolic effects; no effect on peripheral resistance vessels	
	Acetylcholine	in situ	1000–2000 µg	Vasodilation, heart mediated retardation of the pulse frequency	
Octopus vulgaris	Adrenaline	in vivo	10–60 µg kg^{-1}	Systolic and diastolic rise in pulse and pressure	Wells and Mangold[72]
		in vitro	100 µg	Vasoconstriction	
	Tyramine	in vivo	3–40 µg kg^{-1}	Vasodilation	
		in vitro	10 µg	Vasodilation partly followed by a vasoconstriction	
	Serotonin (5–HT)	in vivo	4 µg kg^{-1}	Increase in pulse amplitude and mean pressure	
		in vitro	2 µg	Vasoconstriction followed by a vasodilation	
	Acetylcholine	in vivo	50–100 µg kg^{-1}	Vasodilation	
		in vitro	20 µg	Vasodilation	
	FMRF amide	in vivo	1 µg	Increase of systolic and diastolic pulse pressure	Wells[71]
Sepia officinalis	Noradrenaline	in vitro	10^{-7}–10^{-5} M	Vasoconstriction	Diekmeier
	Adrenaline	in vitro	10^{-7}–10^{-5} M	Vasoconstriction	Schipp 1986 (unpublished)
	Dopamine	in vitro	10^{-7}–10^{-5} M	Vasoconstriction	
	Isoprenaline	in vitro	10^{-7}–10^{-5} M	No effect	
	Serotonin	in vitro	10^{-7}–10^{-5} M	Vasodilation	
	Acetylcholine	in vitro	10^{-7}–10^{-5} M	Vasodilation (?)	
	FMRF amide	in vitro	10^{-5} M	Vasoconstriction	

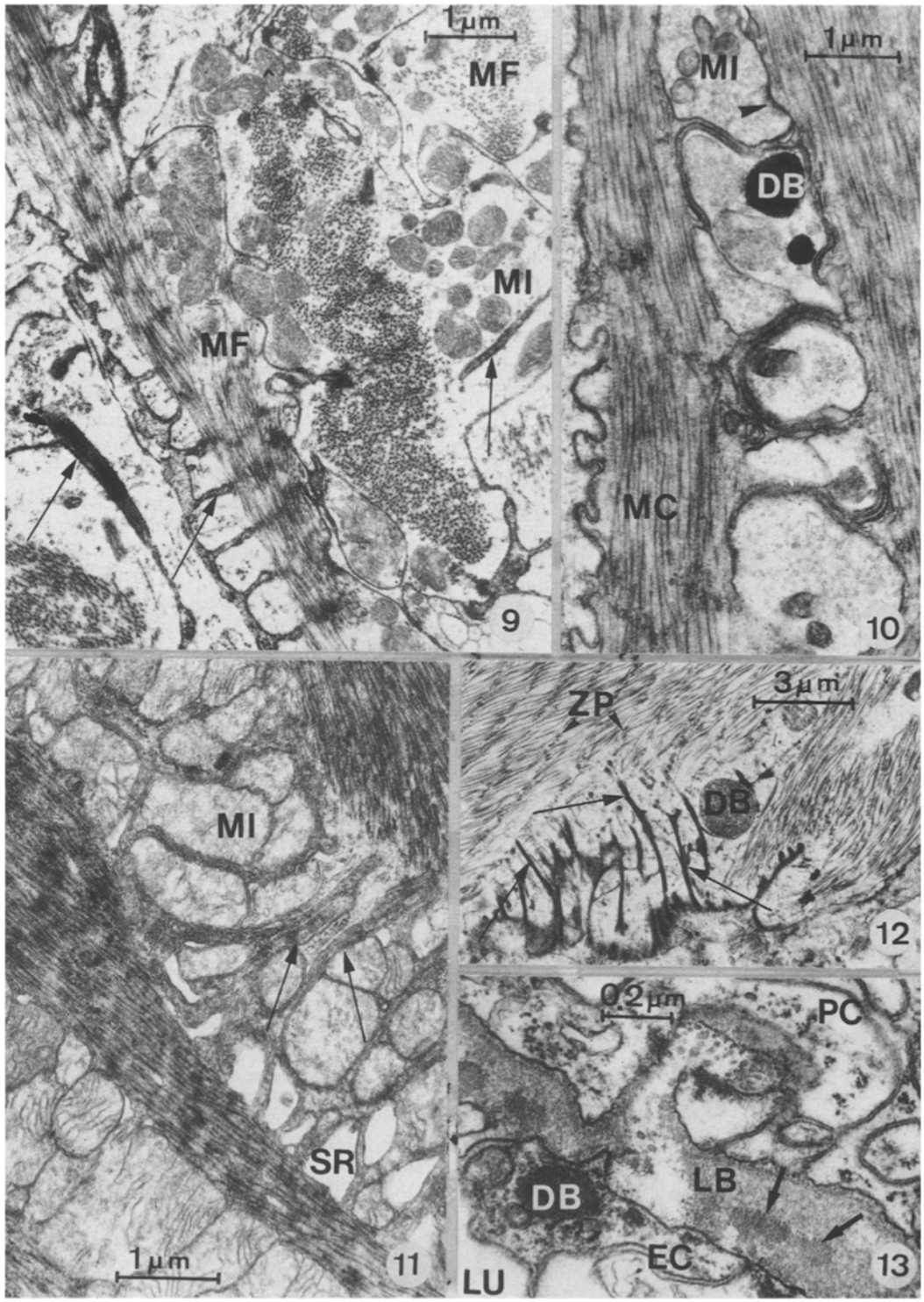


Figure 9. Obliquely striated muscle cells from the T. media of the branchial vein (= efferent branchial vessel) of *Sepia officinalis* in a TEM section; note the numerous mitochondria (MI), the multidirectionally arranged myofilaments (MF), and the t-tubuli (arrows).

Figure 10. Neuromuscular synapsis from the T. media of V. cephalica of *Sepia officinalis;* assembly of small synaptical vesicles on the presynaptic membrane (arrow heads), mitochondria (MI), dense bodies (DB) muscle cell (MC).

Figure 11. Sarcosome (MI) rich muscle cells with close interdigitation (arrows) from the branchial vein of *Ocotpus vulgaris;* enlarged SR tubules (SR) in contact with the t-tubules.

Figure 12. Marginal area of an obliquely striated muscle cell of the *Sepia* branchial vein with few sarcosomes but a distinctly marked system of t-tubules (arrows) on the level of z-patches (ZP), lysosomal dense bodies (DB).

Figure 13. T. intima of the branchial vein of *Eledone cirrhosa* particular dense bodies (DB) seem to be discharged from the endothelial cell (EC) into the matrix of the lamina basalis (LB); note the dense patches there (arrow); pericyte (PC), lumen (LU).

processes[20] could possibly contribute to the firmness and vasodilatation of these veins.

Nervous supply and control of the vessels

The terminal nerves within the vessel wall described in the preceding chapter are parts of an extended peripheric nerve plexus[39,68] (fig. 21). Like the corresponding plexiform nerve arrangements of the hearts (see Kling and Schipp in this review) or the gastric system[76], they are more or less free of ganglion cells and originate from large nerve trunks (for example visceral, sympathetic, posterior and anterior funnel nerves), to some extent with subordinated peripheric ganglions (cardiac, gastric, branchial ganglion)[1].

Investigations as to the course and origin of these nerves that accompany vessels have hitherto mainly concentrated on the V. cava system of coleoids innervated by visceral nerves or 'anterior V. cava nerves'[1,2,4,76,77]. According to them, the origin of these nerves, and also the centers of experimentally proved cardiovascular regulation mechanisms[31,58,69], are generally to be found in the pallio-visceral lobus of the subesophageal brain mass (*Octopus vulgaris*[77], *Nautilus*[75]). This also accommodates other 'lower vegetative centers' (e.g. the controlling center of the respiratory movement) which explains the relative reflex coordination between breathing and cardiac activity, aortic pulse, or arm vein peristalsis observed by different authors[21,30,31,47,58,69].

Although no specific chemo- or pressoreceptor cells have yet been localized within the vessel wall, experimental results on reflex right-left-sensitivity to circulatory activities, or reflex sensitivity of pulsating organs to experimental mechanical stimulations, reveal a high probability that a motor innervation as well as a nervous afference exists, possibly effected by sensitive free nerve endings. That means that reflex mechanisms switched by the central nervous system control the vascular activities (vasodilatation or vasoconstriction)[21,30,58].

What are the transmitters of the vessel nerves?

Apart from the quoted cytological results, according to which both transparent and dense-cored synaptic vesicles appear in the terminal axons of the vessel wall (figs 8 and 10), there are also some histochemical indications for a dual innervation of the obliquely striated muscle cells of the cephalopod arteries and veins. By means of the Falck-Hillarp method and its different modifications, a formaldehyde or glyoxylacid-induced fluorescence microscopical reaction to prove the existence of catecholamines and the indolealkylamine, 5HT, succeeded in establishing the existence of aminergic nerves, especially in the T. adventitia of the pharyngeal and visceral arteries[6,22] as well as in the afferent branchial vessels or the aorta cephalica and aorta abdominalis[39]. Similar observations of a green-yellow fluorescence are available for the arm veins and V. cephalica of *Eledone moschata*[68] and *Sepia officinalis* (Schipp 1984, unpublished). Such aldehyde-induced monoaminergic fluorescences were also seen in the vasomotoric tract of the palliovisceral lobus and the visceral nerves of the *Octopus* brain[64] or ganglion cardiacum of *Sepia*[40]. A significant point is the regularly observed aldehyde-independent fluorescence of larger, not serially arranged particles (∅ 0.1–1 µm), like those also observed in heart nerves of coleoids[36-38]. They are obviously to be assigned to the above-mentioned glial dense bodies and are considered to be metabolic wastes by some authors[37]. The generally very high MAO activity in the muscle cells, nerve trunks and endings of the coleoid vessels (V. cephalica, arm veins, efferent and afferent branchial vessels, aorta[39,68], Schipp 1984 unpublished) (fig. 18) corresponds

Table 2. Effects of neurotransmitter substances on the pulsatile coleoid veins

Species	Vessel type	Substances	Methods of application	Applied doses	Responses		References
Octopus vulgaris	Arm veins	Adrenaline	in vitro	$1:10^5$	Systolic effects, no changes of pulse frequency		Mislin and Kauffmann[48]
	Arm veins	Acetylcholine	in vitro	$1:10^5$	Induction of peristalsis in inactive vessels, positive chronotropic and inotropic effects		
Octopus dofleini	Efferent branchial vessel (= branchial vein)	Acetylcholine	in situ	500 µg	Increase of the pulse pressure, positive inotropic, negative chronotropic		Johansen and Huston[30]
			in situ	2000 µg	Temporary standstill arrhythmical contractions		
		Serotonin (5–HT)	in situ	100 µg	Positive inotropic, no chronotropic responses		
	Vena cephalica	Acetylcholine	in situ	500 µg	Increase of the pulse pressure after longer filling times and an increased distension		
		Histamine		1000 µg	A short acting interference with a general drop in pressure		
Sepia officinalis	Vena cephalica				PLM	CMM	
		Adrenaline	in vitro	10^{-9}–10^{-6} M	− inotropic − chronotropic	No peristalsis No peristalsis	Schipp and Schuck 1985/86 (unpublished)
		Serotonin (5-HT)	in vitro	10^{-9}–10^{-5} M	− inotropic − chronotropic	No peristalsis	
		Acetylcholine	in vitro	10^{-9}–10^{-4} M	+ inotropic + chronotropic	Peristalsis Peristalsis	
		Nicotine	in vitro	10^{-9}–10^{-5} M	+ inotropic + chronotropic	Peristalsis Peristalsis	
		FMRF amide	in vitro	10^{-7}–10^{-6} M	No effect	No effect	

to these demonstrations of monoamines. There is accordingly no doubt that, as is the case with heart organs (see Kling/Schipp and Fiedler/Schipp in this multi-author review) the blood vessels of coleoids also have an aminergic innervation (for *Nautilus* there are as yet no results available). The question of exactly which amines occur, however, remains open. Candidates are dopamine, tyramine, noradrenaline and 5HT (serotonin), which

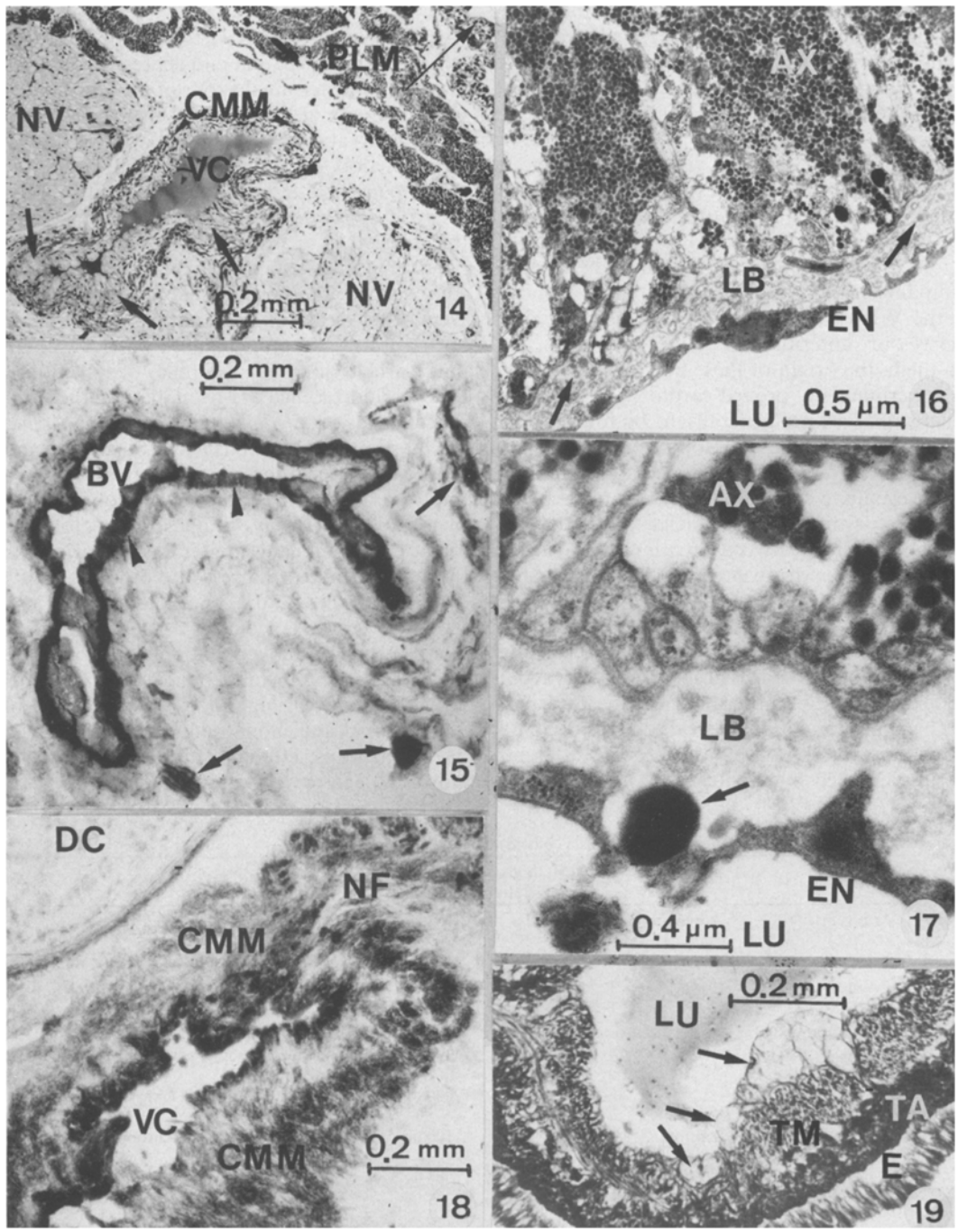


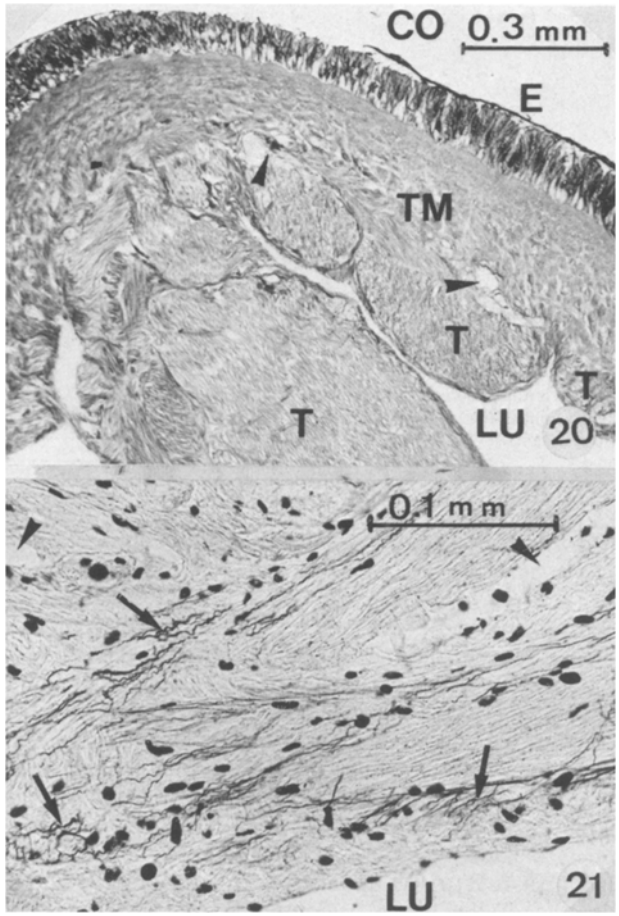
Figure 14. V. cephalica (VC) of *Sepia officinalis* after the passing of n. visceralis (NV) through the foramen of the diaphragm in a LM section; periadventitial longitudinal muscles (PLM), T. media circular muscle (CMM), epithelium of the mantle cavity (long arrow), NSV system (short arrows).

Figure 15. AChE-reaction in the T. media muscle of the pulsatile branchial vein (BV) of *Sepia officinalis* (arrow heads) and its supplying nerve trunks within the T. adventitia (arrows) (from Wecker[68]).

Figures 16 and 17. NSV area of the V. cephalica of *Eledone cirrhosa*. Note the osmiophilic granules of different diameter within the enlarged terminal axons (AX) and their exocytotic releasing (arrows) via the lamina basalis (LB) and the endothelium (EN); lumen (LU).

Figure 18. Strong MAO reaction within the nerve fibers (NF) and terminal axons (arrows) of the NSV-system of the *Sepia* V. cephalica (VC); note the weaker reaction within the CMM; diaphragm cartilage (DC).

Figure 19. LM section of the branchial vein of *Nautilus macromphalus;* enlarged terminal axons (arrows) near the lumen indicating a 'NSV-system'; T. media (TM) with longitudinal and circular muscle fibers; collagenous fibers of the T. adventitia (TA), epithelium (E).



Figures 20 and 21. LM sections of the pulsatile V. cephalica of *Nautilus macromphalus;* note the strong differently arranged muscle fibers of the T. media (TM) and their trabecular (T) structure in the luminal area (LU); intramural vasa vasorum and collecting sinus (arrow heads), coelom (CO), epithelium (E); in fig. 21: a Bodian silver staining of the very dense peripheral nerve plexus (arrows) of the vessel wall.

were also established biochemically to be present in the vasomotor lobes of the subesophageal brain of coleoids[33, 34, 63, 64].

The histochemical indications for a cholinergic nerve supply of the vessel wall (comparable with that of the hearts) working antagonistically to the aminergic nerves, having mainly been based so far on the localization of acetylcholinesterase (AChE) (EC 3.1.1.7). High levels of activity for this enzyme have thus far been found in the mentioned nerve trunks, especially the nervus visceralis, also in the nerve plexus, the muscle cells and the lamina basalis of the vein wall (fig. 15) (V. cephalica, arm veins, efferent branchial vessels of coleoids[68] and vessels of *Nautilus* gill[50]) and the arteries examined thus far (aortae, afferent branchial vessels of octopods)[38]. The AChE localization in different areas of the central nerve system of *Octopus vulgaris*[12] and *Sepia officinalis*[19] as well as the subesophageal mass of *Eledone cirrhosa*[63, 64] is also an indication for the fact that ACh acts as a physiological transmitter in the vessel motoric. Immuncytological localization and biochemical identification of peptides within the nerve endings of the vessel wall tend to speak increasingly against a purely dual conception of an antagonistic monoaminergic-cholinergic vasocontrol. In this connection the findings on the above-mentioned neurosecretory veins, the 'NSV-system', of coleoids should first be stressed[41–43, 66] and they are the subject of a separate contribution by the present authors in this multi-author review.

The physio-pharmacological aspects of the vascular regulation

The pharmacological findings on the vessels are not very numerous, unlike those relating to the cephalopod heart. Moreover, the investigations have seldom been performed on isolated vessel segments[46, 48, 72] but more frequently in vivo, on the more-or-less intact circulatory system of anesthetized animals[30, 72]. When applying drugs, the authors were therefore often not able to come to a clear decision about whether myovasal receptors could be regarded as the site of a pharmacological effect, or whether perhaps the registered vessel reaction took place indirectly by interfering effects on adjacent heart organs or humoral stimulations in the CNS.

The investigations on arteries undertaken so far have concentrated mainly on the cephalic aorta and to a lesser extent the branchial vessel and only refer to coleoids.

The results summarized in table 1 show that both in vivo and in vitro experiments with acetylcholine show relatively uniformly a vasodilatative effect. In contrast, the vasoconstrictory effect of catecholamines is not confirmed by all experiments. The depressor effects on the cephalic aorta of *Octopus dofleini*[30] form a special contrast to those of an increase of the vessel tonus in *Octopus vulgaris*[71, 72] and *Sepia officinalis* (fig. 22). Results on the 5-HT effect are also contradictory, as both an increase of the systolic pressure and vasodilatation were observed. The investigations testing the effect of cardio- or vasoactive peptides on the tonic muscle of the cephalic aorta are still in their initial stages. Preliminary results indicate that

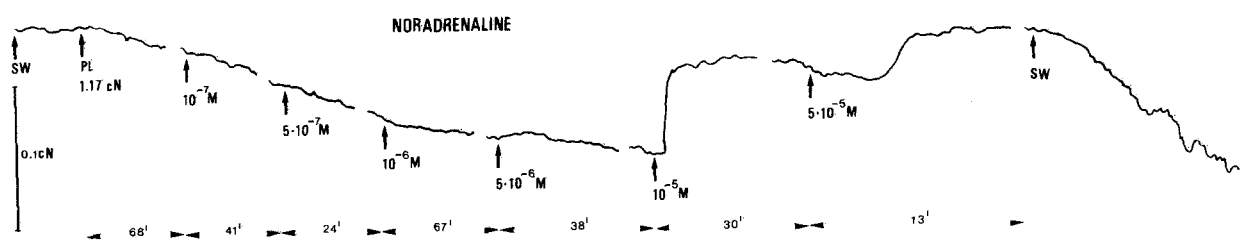


Figure 22. Isometric contractile responses of the perfused cephalic aorta (flow rate: 0.3–0.5 ml/') of an adult *Sepia officinalis* (ML 14 cm) to cumulatively increasing concentrations of noradrenaline in the perfusing solution; preload: 1.17 CN; note the continuous extension tendency during the perfusion with seawater-glucose solution and lower drug concentrations (Diekmeier and Schipp, unpublished).

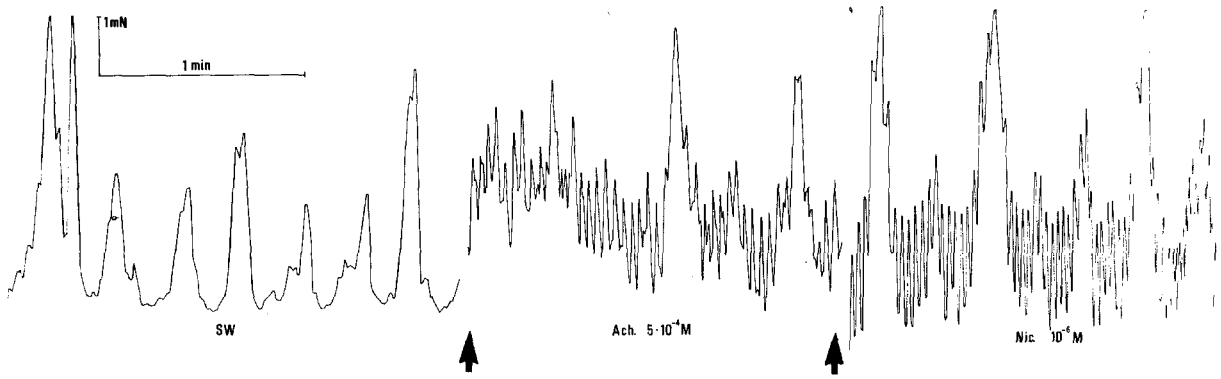


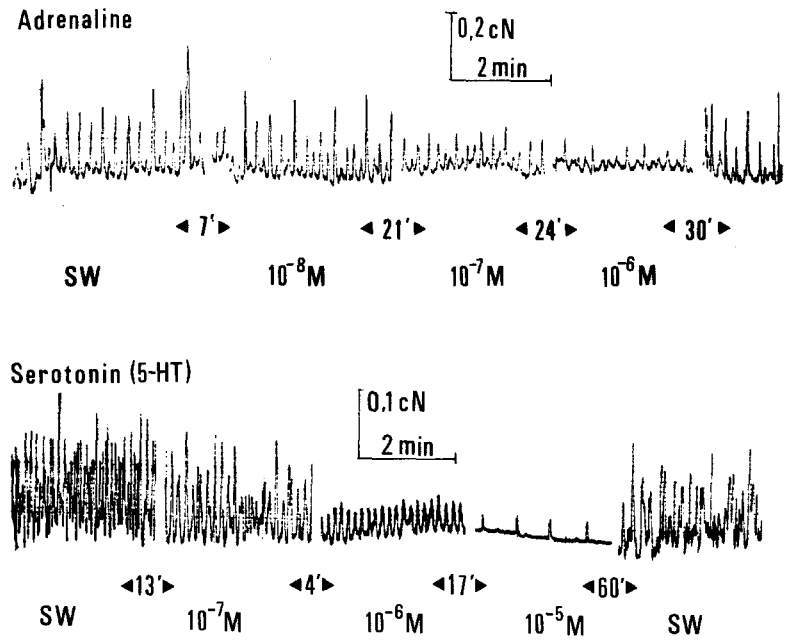
Figure 23. Effects of Ach and nicotine (Nic) on the autonomous pulse activity of the isometric suspended and perfused cephalic vein of semi-adult *Sepia officinalis;* under seawater-glucose perfusion, only the PLM shows rhythmical contractions; Ach and Nic perfused in threshold concentrations induce the peristalsis of the CMM (arrows) (further explanations in text).

FMRFamide – also proved to be present in the NSV-layer of coleoids[66] – has a vasoconstrictory effect on the cephalic aorta of *Sepia officinalis*. That means that, possibly just as in the systemic heart of coleoids[71], (see contribution Kling/Jakobs in this review) receptors for these peptides are present and thus the central heart and peripheric resistance vessels react synergically to a releasing of NSV-peptides; however, angiotensin II, an extremely potent contractile stimulant of the vascular smooth muscle in vertebrates, had no effect.

Pharmacological results on peripheric arteries are even fewer in number. They are restricted to some in vivo experiments on the afferent branchial vessel of *Octopus dofleini*[30], where injections of 5-HT (100 µg) and adrenaline (300 µg) led to a systolic increase of pulse pressure; on the other hand, a smaller dose of the latter transmitter (50 µg) led to decreasing effects.

The myogenic automatism and the neuro-humoral control of the pulsatile veins

As it has already been pointed out above, the pulsatile veins found in different parts of *Octopus vulgaris*[23] are one of the most remarkable pecularities of the dynamic circulatory system of nautiloids or coleoids. Hemolymph from the named blood sinus, capillary beds and smaller collecting veins is conveyed back to the central heart organs by this vein peristalsis. Following Starling's law it adapts to the blood volume flowing in from the periphery in its metachronic pulse[21, 58, 67] and pulse pressure, thus having a direct effect upon stroke volume and the rate of beating of the hearts[60]. Compared to the arteries, its systolic pressure shows distinctly smaller values, also greater fluctuations, and the diastolic pressure is often near the zero level[30, 31, 58] (compare table 1 of the introduc-



Figures 24 and 25. Negative inotropic effects of adrenaline and 5-HT on the PLM activity of the perfused cephalic vein of a semi-adult *Sepia officinalis;* the peristalsis of the CMM could not be induced by catecholamines or indolalkylamines (further explanations in text).

tory paper). With his experiments on the isolated arm vein of *Octopus vulgaris* Mislin[45,48] first proved that these vessels, similarly to the hearts, have a myogenic automatism whose peristaltic frequency (in situ: 20–30/′ at 17 °C) and stroke volume depend on the temperature (6°–26 °C→6–130/′) and hydrostatic pressure (2–10 cm H_2O→3–11/′ at 17 °C); however, in situ there is a reflex nervous control by the subesophageal CNS. The latter could be well confirmed by simultaneous in situ pressure measurements on the arm and cephalic veins in *Octopus dofleini*[30,31,58]. Furthermore these authors indirectly proved a more complex mechanism of reflex regulation with different only phasic synchronized working inhibitory and excitatory centers of the subesophageal brain; these authors have already reported on a relative dependence of the pulsatile activity on the respiratory movement of the mantle.

Our physiopharmacological experiments on ortho- and retrograde perfused V. cephalica of *Sepia officinalis* focussed on the isolated vessel segment between the passing site of the N. visceralis through the diaphragm cartilage and the V. cava bifurcation, which also contained parts of the 'NSV system'[4].

The results revealed that the activity of the V. cephalica is based on two muscle systems[4] that are relatively independent of each other (fig. 14); firstly, autonomous contractions of the very strong periadventitial longitudinal muscle layer (PLM) (orientated towards the mantle cavity); secondly, the peristalsis of the circularly arranged inner vessel muscle of the T. media (CMM) characterized by a particularly high content of sarcosomes and a high density of terminal nerves.

With isometric suspension and adequate physiological filling pressure of this vessel preparation only the PLM showed a spontaneous and autorhythmical contractility. In contrast to Mislin's findings on the isolated arm veins of *Octopus*[45,48], peristaltic pulses of CMM could not be triggered under normal physiological conditions or even with higher filling pressures. When the vessel segment was perfused with ACh-seawater-solution however, (cumulative application 10^{-9}–10^{-4} M), at lower concentrations only the contractions of the PLM were positively influenced inotropically, depending on the dose, yet hardly influenced chronotropically. After a threshold concentration of 10^{-5}–$5 \cdot 10^{-5}$ M ACh had been reached, peristaltic pulse waves of the CMM did begin; this corresponded to the findings on the isolated arm veins in *Octopus*[45,48]. These waves could be increased in frequency and amplitude, depending on the dose, within a limited concentration range (10^{-5}–10^{-4} M). At values of 10^{-4} M, however, systolic hypercontractions with standstill were mostly the result, but these were reversible, i.e. could be cancelled out after rinsing for a long time with seawater glucose solution (fig. 23). Experiments with different agonists and antagonists revealed that this effect can be mimicked by nicotine, with a 10^1 lower threshold concentration (10^{-6} M) (fig. 23), but not muscarine, so that there seems to be a nicotinic receptor, similar to that in systemic and branchial hearts of coleoids (see Kling/Jakobs and Fiedler/Schipp in this multi-author review). This marked ACh-sensitivity of the V. cephalica corresponds to the above-mentioned AChE (E.C.3.1.1.7) activity, which characterizes both muscle layers and their supplying nerves but not, however, the luminal nerve terminals of the 'NSV-system'.

As shown by table 2, the vessel segment is insensitive to FMRFamide, the cardioactive peptide that was found in the 'NSV-system' of octopods[66] (see above) and is probably also released in this vessel segment of *Sepia officinalis* without affecting it.

According to first results, and in contrast to the in situ observations of the efferent branchial vessels of *Octopus dofleini*[30], adrenaline and 5-HT have a negative inotropic effect (figs 24 and 25). This result corresponds with TEM findings of dense-cored synaptic vesicles in terminal axons, as well as with histochemical results showing a high MAO-activity (fig. 18) and formaldehyde-induced fluorescence especially of the inner muscle layers (CMM) and their nerves. Accordingly both substances can be considered as being ACh antagonistic also in the pulsatile veins, i.e. can be regarded here – to a certain extent in contrast to their effects on arteries – as inhibitory neurotransmitter candidates.

To sum up we can conclude from the available results that the autonomous contractility of the coleoid veins is based on a myogenic component. But the typical peristalsis of the V. cephalica is obviously mediated neurogenically in vivo by the release of ACh from cholinergic nerve terminals. Further investigations will be needed to characterize the membrane receptors of myocytes more closely, and also to investigate other potential transmitter candidates (dopamine, GABA); on the other hand, it remains to be seen whether the depolarization of the sarcolemma, induced 'spontaneously' or by ACh stimuli as in cardial pacemaker myocytes, primarily takes place via the mechanism of an increased abbreviation of the repolarizing K^+ permeability on the ligand-gated cation channels. The positive inotropic effects mentioned are, as in cardiomyocytes, most probably also in veins the result of an increased inward current of Ca^{++} (compare the TEM results on the differentiation of a t-system) intensifying the electromechanical coupling.

Acknowledgment. We would like to thank the Deutsche Forschungsgemeinschaft for its support, Dr C. Cazaux, who provided the facilities for work in the Station de Biologie Marine Arcachon and G. Balser, S. Beckermann, A. Hudel, M. Kopsch, J. Luh and H. Schmidt for their technical help.

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0014-4754/87/050525-13$1.50 + 0.20/0
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The neurosecretory system of the octopus vena cava: A neurohemal organ

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Summary. Endings of about two million small neurones form a voluminous neuropil inside the vena cava of cephalopods, in direct contact with the blood. These nerve endings are filled with masses of typical neurosecretory granules. By immunocytochemistry we could distinguish three different populations of secretory endings in the vena cava neuropil of *Octopus vulgaris:* 1) a population of endings which were immunoreactive with antibodies against the pentapeptide proctolin; 2) a population with oxytocin/vasopressin- and neurophysin-like immunoreactivity; 3) a population immunoreactive with antibodies which were raised against the molluscan cardioexcitatory peptide Phe-Met-Arg-Phe-amide, against α-melanotropin, and against atriopeptin. Extracts of the octopus vena cava stimulated amplitude and frequency of the isolated octopus heart preparation. Similar effects were exerted by peptides with the C-terminal structure -Arg-Phe-amide. Recently, we could isolate and identify in vena cava extracts four peptides; Phe-Met-Arg-Phe-amide, Phe-Leu-Arg-Phe-amide, Ala-Phe-Leu-Arg-Phe-amide and Thr-Phe-Leu-Arg-Phe-amide. Other peptides have not yet been identified. The fact that the peptides against which the immunoreactive antibodies were raised affected, in different organisms, blood volume, blood pressure, renal function and heart contraction suggests that one of the main functions of the neurosecretory system of the vena cava is a hormonal control of circulation.
Key words: Octopus circulation; vena cava neuropil; neurosecretion; Phe-Met-Arg-Phe-amide-like peptides.

Introduction

Cephalopods have been known for some time to possess an organ which in some respects resembles the neurohypophysis of vertebrates. It is the neurosecretory system of the vena cava (NSV-system), the structure and histology of which has been thoroughly described by Alexandrowicz [2, 3]. The NSV-system, due to the position of its nerve endings inside the vena cava in direct contact with the blood stream, and to the presence of substances in the nerve endings which control heartbeat and circulation, is an intrinsic part of the cephalopod circulation.

Immunocytochemical methods allowed the distinction of different populations of secretory nerve endings in the vena cava neuropil. Antibodies against smaller peptides which are known to affect blood circulation in cephalopods and other organisms were immunoreactive in the secretory endings. Some of the antigenic cephalopod peptides have now been identified. It is our aim to summarize current knowledge about the bioactive peptidic agents in this organ. This review specifically refers to *Octopus vulgaris* from the Bay of Naples and from Banyuls-sur-Mer. There are other similar organs which apparently control cephalopod circulation, especially a system discharging into the pharyngo-ophthalmic vein[9], or tissues connected to sinuses in the head cavities (see Young[34] for a review). Also, in oegopsid squids NSV-neurones forming a voluminous glandular tissue in the palliovisceral ganglion appear to release their products directly into capillaries of the brain[14]. To our knowledge no further data are available on these organs.

An outline of the histology of the NSV-system

A most detailed description of the different elements of the NSV-system in *Eledone*[2], *Sepia* and *Octopus*[3] is due to J.S. Alexandrowicz. We should like to introduce the essential parts of this system.

The most conspicuous part of the NSV-system is a voluminous neuropil layer which forms the inner surface of