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Cephalopod myocardial receptors: Pharmacological studies on the isolated heart of *Sepia officinalis* (L.)

by G. Kling and P. M. Jakobs

Institut für Allgemeine und Spezielle Zoologie, Justus-Liebig-Universität, Stephanstr. 24, D-6300 Giessen (Federal Republic of Germany), and Station de Biologie Marine d'Arcachon, F-33120 Arcachon (France)

Summary. This paper presents a synopsis of the information available about the pharmacological action of various substances on the cephalopod heart, with special emphasis on the central heart of *Sepia officinalis*.
Threshold concentrations, EC_{50} values and maximum effective concentrations have been experimentally determined. Studies with various transmitter substances, analogous compounds and antagonists have led to the following picture: Acetylcholine is the natural inhibitory transmitter substance; it acts via receptors with nicotinic properties which can be blocked by d-tubocurarine and α-bungarotoxin. The probable excitatory transmitter system is represented by a noradrenergic innervation. Noradrenaline has a positive inotropic and a positive chronotropic action on in vitro heart preparations. A positive inotropic response can also be evoked by serotonin (5-HT); this effect is not due to stimulation of the catecholamine receptor, as is shown by cross-over experiments with specific blocking agents. Furthermore, a peptidergic receptor system has been described which reacts with the 'molluscan cardioactive peptide' FMRF amide most effectively. It is assumed that cardioactive peptides may reach the central heart in the circulating blood; the sites of synthesis and release are still unknown. Possibly the NSV-layer of the vena cava is involved in hormonal cardiovascular regulation processes.
Key words. Cephalopods; central heart; pharmacology; receptors; acetylcholine; catecholamines; indolalkylamines; peptides.

General remarks

Pharmacologically active substances whose mode of action is known are often used as tools to aid research into physiological principles that are still unknown. For investigation of receptor-mediated processes it is important not only to test the effects of one drug concentration, but also to record the whole dose range from the threshold concentration up to the maximum effect, because unphysiological concentrations may cause atypical effects, depending on the sensitivity of the investigated organ preparation.
Only by means of dose-dependent effects, demonstrated by a sigmoid dose-response-curve (DRC) or concentration-response-curve (CRC) and supported by special inhibition experiments with antagonists, can a receptor stimulation be suggested.
Isolated myogenic hearts are very suitable for investigations using pharmacological methods, owing to their rhythmic activity. This provides a simple method of measurement for control and evaluation. Concentrations of active substances, and amplitude and frequency values can be recorded without much trouble.
Studies with isolated organs have various advantages compared with those on intact animals. For example, the reaction of one muscle type to the drug can be measured (this applies especially to strip preparations) without the interference of nervous regulation and counterbalance. The receptor equipment and sensitivity of an isolated muscle can thus be well established. These preparations are easily accessible for direct measurements. Furthermore, animal movements, which would otherwise the troublesome, are also excluded. The disadvantage of the in vitro technique is that such experiments only produce results for a model preparation; the conclusions cannot be transferred directly and uncritically to the intact animal, and do not represent the normal physiological relationships.
Depending on the formulation of the question, scientists must decide on the one or the other method. The two methodological possibilities do not compete, but rather complement one another in the solution of special scientific questions.
In in vitro studies on cephalopod hearts the reflow system with a Straub cannula has been most frequently used[57, 59]. This system, which was also usually applied in our investigations, has some disadvantages in comparison with continuous perfusion techniques. The flow direction of artificial blood fluid does not represent the physiological circumstances; furthermore, incubation fluid is not renewed continuously, and especially when using inhibitory substances, mixing with the incubation fluid may be inadequate[57]. On the other hand, an exact definition of pre- and after-load pressure and a better control of temperature is possible when using the perfusion technique. In spite of the above-mentioned qualitative differences,

we obtained identical results with both methods. This may correlate with the fact that in the *Sepia* heart a coronary system is virtually negligible, whereas in *Octopus* hearts this vascular system is more highly developed (see also foregoing article). Cutting the venae cordis for the isolation may have some influence on the heart activity in octopods[33].

The following overview is divided into the main topics acetylcholine, catecholamines, serotonin and peptides. Earlier work is considered in the text only insofar as noteworthy results were obtained by the authors. Otherwise the articles of Krijgsman and Divaris[61], Fischer[28], Richter[79] and Leake and Walker[66] form the basis for reviewing recent results.

The acetylcholine (ach) orbit

Acetylcholine the 'Vagusstoff', first described by Loewi[68], normally serves to transmit nervous excitement from nerves to response organs in the animal organism. This process is connected with changes in ion permeability and is considered to be receptor-mediated. But ach is also found in non-innervated organs and can evoke effects there[36, 88]. This indicates that neither the biochemical demonstration of ach nor its pharmacological effect is sufficient alone as proof of a cholinergic innervation.

It is the demonstration of the presence of ach, its pharmacological effects, and the enzymes of ach-synthesis and catabolism that should form the basis for continuing investigational methods, like in vitro-binding studies, intraaxonal demonstration of transmitters, receptor visualization by fluorophore-coupled antagonists, electrophysiological methods with ion-sensitive electrodes, etc.

Only if an innervation is ascertained by histology or cytology, and if no less than two of the above-mentioned methodological criteria are fulfilled, and a positive result is obtained by these methods, can a cholinergic innervation be postulated. Up to now ach has been found in nearly all animal species and therefore it seems to be an ancient principle of cellular communication. The reasons for this may be: 1) acetylcholine can be synthetized with one enzymatic step from choline and acetate, 2) with simple enzymatic hydrolysis the molecule is decomposed into pharmacologically inactive primary products again

Table 1. The action of acetylcholine on the cephalopod heart

Species	Acetylcholine concentration	Effect	Preparation and mode of drug application	References
Sepia officinalis	0.05 mg	Negative inotropic, positive chronotropic	Isolated heart	62, 65
	0.05 mg	Negative inotropic, negative chronotropic or cessation for a few seconds		
	1 mg	Cessation for one minute, then postinhibitory overshooting		
	1–2 mg	Cessation for a few minutes, then post-inhibitory overshooting		
Sepia officinalis	10^{-6}*	Cessation for 10 s	Isolated heart, application by injection into perfusion fluid	29
	10^{-5}*	Cessation for 30 s		
Sepia officinalis	$< 10^{-7}$ mol/l	No effect	Isolated heart (Straub cannula) application by perfusion with drug-seawater solution	57
	5×10^{-7}– 2×10^{-5} mol/l	Negative inotropic, negative chronotropic		
	6×10^{-5} mol/l	Cessation for 30–60 s, then postinhibitory overshooting		
	$> 5 \times 10^{-5}$ mol/l	Total irreversible cessation $EC_{50} = 10^{-5}$ M		
Loligo pealii	10^{-8}*	Negative inotropic, negative chronotropic or cessation	Isolated heart	10
Loligo pealii	10^{-9} (approx.)*	Negative inotropic, negative chronotropic	Isolated heart, application by giving droplets of 10^{-4}* solution	11
	10^{-7} (approx.)*	Cessation		
Octopus dofleini	100–2000 µg	Negative inotropic, negative chronotropic	In vivo, application through an implanted catheter into the efferent branchial vessel	47
Octopus vulgaris	10^{-7}*	No effect	Isolated heart	35
	10^{-6}*	Negative inotropic, negative chronotropic		
	5×10^{-6}*	Cessation for a few seconds		
	5×10^{-5}*	Cessation for a few minutes, then post-inhibitory overshooting		
Octopus vulgaris	20–100 µg/kg	Negative inotropic, negative chronotropic, then postinhibitory overshooting	In vivo, application into gill heart	91
	15–50 µg/kg	Negative inotropic, negative chronotropic, then postinhibitory overshooting	Application into the efferent branchial vessel	
	10–20 µg/heart	Negative chronotropic, cessation for 10–20 s	Isolated heart, application into the perfusion fluid of the leading-in catheter	
Octopus vulgaris	10 µg	Inhibition	Isolated perfused heart, application by injection into perfusion fluid	91
	20 µg	Cessation for 10 s followed by increased amplitude and frequency		

* No declaration of unit.

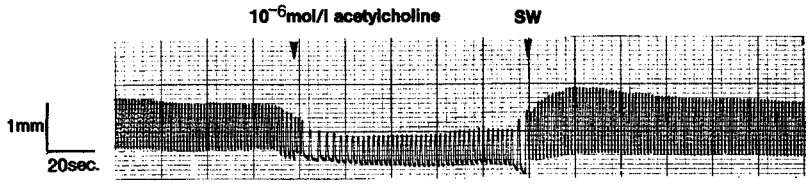


Figure 1. The action of 5×10^{-6} mol/l acetylcholine on the isolated *Sepia* heart, demonstrating a strong decrease of amplitude and frequency (from Kling[57]).

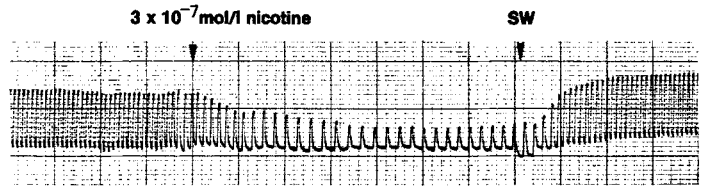


Figure 2. Nicotine (3×10^{-7} mol/l) mimics the acetylcholine response but acts at lower drug concentrations than acetylcholine (from Kling[57]).

and 3) acetylcholine is a very polar substance which is very suitable for interactions with complementary structures (receptors!)[66].

Bacq[10, 13] and Kruta[63–66], the pioneers of invertebrate pharmacology, also performed basic work on the action of ach on the cephalopod heart. Together with recent results, their work leads to the conclusion that acetylcholine effects a reduction of contraction amplitude and frequency values in the isolated heart of *Sepia officinalis*.

In all experiments performed by different authors such an inhibition was found[10, 11, 29, 65]. The only exception was reported by Kruta[63], who found a slightly positive chronotropic effect when adding a few crystals of ach (0.05 mg) to the incubation medium. The threshold concentration for the inhibitory effect on the isolated *Sepia* heart was about 10^{-7} mol/l. Using increasing ach-concentrations up to 10^{-6} mol/l ach, a transient reduction of concentration amplitude and frequency occurred. After not more than

Table 2. The action of cholinomimetic substances on the cephalopod heart

Species	Substance concentration	Effect	Preparation and mode of drug application	References
	Nicotine			
Octopus vulgaris, Loligo vulgaris, Ommastrephes	1:1000 and 1:10000	First acceleration, then cessation	Isolated heart 1. Application on the surface of the heart 2. Application into the heart lumen through a cannula	21
Sepia officinalis and *Octopus vulgaris*	0.01–0.1 mg 10^{-7}*	Cessation Inhibition	Isolated heart, application by perfusion	64
Sepia officinalis	$< 10^{-9}$ mol/l 6×10^{-9}–10^{-6} mol/l 6×10^{-6} mol/l $> 6 \times 10^{-6}$	No effect Negative inotropic, negative chronotropic Cessation for 2–5 min, then postinhibitory overshooting Irreversible cessation, acetylcholine-mimicry, $EC_{50} = 6 \times 10^{-7}$ M	Isolated heart (Straub cannula) application by perfusion with drug-seawater solution	57
	Muscarine			
Octopus vulgaris	1–2 mg/cm^3 0.5 mg/cm^3	Cessation Inhibition	Isolated heart	78
Sepia officinalis	10^{-9}–5×10^{-4} mol/l	No effect	Isolated heart (Straub cannula), application by perfusion with drug-seawater solution	57
	Pilocarpine			
Sepia officinalis	10^{-9}–10^{-4} mol/l 4×10^{-3} mol/l	No effect Negative inotropic	Isolated heart (Straub cannula), application by perfusion with drug-seawater solution	57

* No declaration of unit.

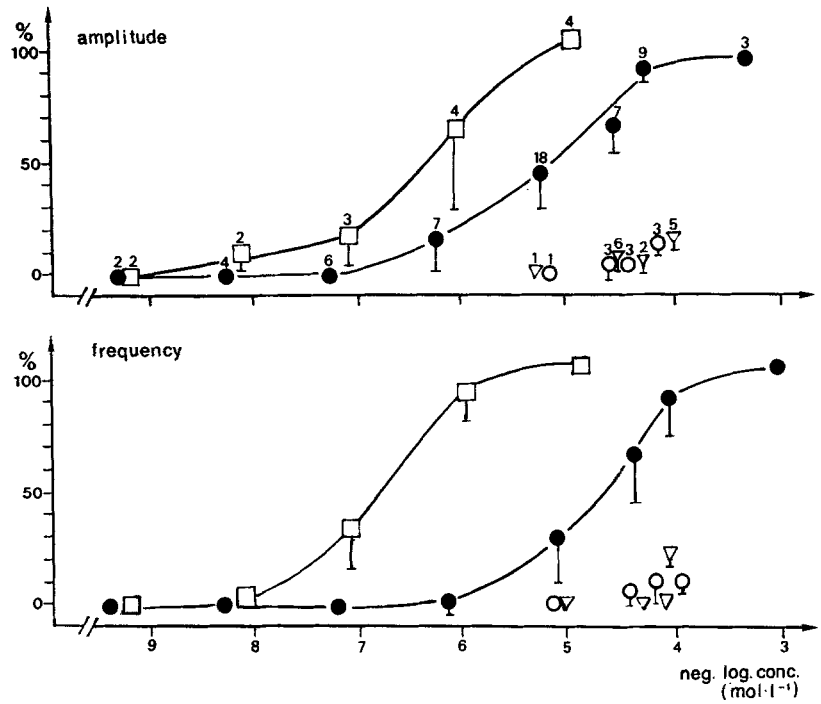
one minute the base values for the untreated organ preparation were reached again. This tachyphylactic effect is probably due to the high activity of cholinesterases demonstrated in the luminal trabecular muscle layer[56]. 5×10^{-6} mol/l ach evokes a sustained inhibition, which is reversible if the drug solution is exchanged for artificial blood fluid (fig. 1). 5×10^{-5} mol/l ach effects an immediate diastolic arrest; after 1–4 min beats start again, aperiodically, and eventually cumulate to a so-called 'postinhibitory overshooting'. After this increased activity amplitude and frequency, values normalize. When the ach-concentration is further increased, a total and irreversible diastolic arrest results. An EC_{50}-value of 10^{-5} mol/l was ascertained for the half-maximal effect[57] (figs 3 and 4). In vitro experiments on octopod hearts revealed corresponding results[35, 47, 91] (table 1). Acetylcholine primarily evokes an increase of K^+, and additionally of Na^+ and Ca^{++} ion permeability, on excitable cell membranes of vertebrates. This counteracts the depolarization and extends the repolarization phase. This mechanism of action may also be assumed for cephalopods, because a resting potential of the Na^+/K^+-type was also formulated for molluscan myocytes[79].

The above-mentioned postinhibitory 'overshooting' in addition to ach-induced inhibition seems to be typical of cephalopod myocardia, as all authors describe this phenomenon. It cannot be excluded that this reaction is caused by contrarotating and regulatory processes in the

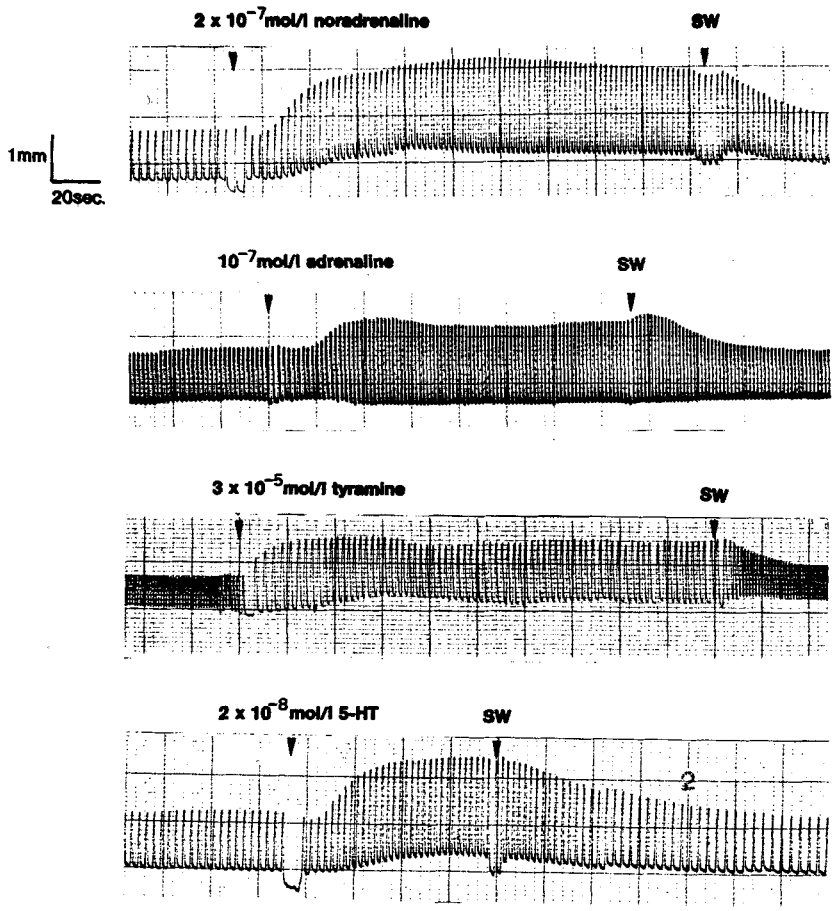
Table 3. The action of cholinergic antagonists on the cephalopod heart

Species	Substance concentration	Effect on the heart	Effect in combination with ach application	Preparation and mode of drug application	References
	Atropine				
Loligo pealii	10^{-6}*	Negative inotropic, negative chronotropic	No reduction of acetylcholine sensibility	Isolated heart	10, 11
	10^{-4}*	Negative inotropic, negative chronotropic	Slight reduction of acetylcholine sensibility		
	1:1000*	Cessation			
Sepia officinalis	0.2 mg	No effect	No acetylcholine antagonism	Isolated heart	63, 65
	0.2 mg	No effect			
Sepia officinalis	10^{-9}–10^{-4} mol/l	No effect	No acetylcholine antagonism, no receptor-blockade	Isolated heart (Straub cannula), application by perfusion with drug-seawater solution	57
Octopus vulgaris, *Loligo vulgaris*, *Ommastrephes*,	1:10000	First acceleration, then inhibition		Isolated heart 1. Application on the surface of the heart	21
Loligo vulgaris	0.5%		Paralyzation of the inhibitory cardiac nerve	2. Application into the heart lumen through a cannula	
Octopus vulgaris	·1 mg/cm^3	Excitation	No paralyzation of the inhibitory cardiac nerve	Isolated heart, application by injection into the heart	78
	Tetraethylammonium				
Sepia officinalis	10^{-9}–10^{-4} mol/l	No effect	No acetylcholine antagonism, no receptor-blockade	Isolated heart (Straub cannula), application by perfusion with drug-seawater solution	57
	Curare				
Octopus vulgaris	*	Excitatory		Injection into the isolated heart	78
Octopus vulgaris, *Loligo vulgaris*, *Ommastrephes*,	1:1000 and 1:10000	First acceleration, then cessation		Isolated heart 1. Application on the surface of the heart	21
Loligo vulgaris	0.5% and 1%	Incomplete tetanus	Blockade of the cardio-inhibitory nerve	2. Application into the heart lumen through a cannula	
Eledone moschata	0.1, 0.5 and 0.001		Blockade of the cardioinhibitory nerve stimulation effect	In situ perfused heart	34
Sepia officinalis and *Octopus vulgaris*	3×10^{-5}*	Negative inotropic, negative chronotropic	Blockade of the inhibitory acetylcholine effect	Isolated heart, application by perfusion	64
Sepia officinalis	10^{-9}–10^{-4} mol/l	No effect	Total reversible blockade of the acetylcholine receptor (10^{-4} mol/l)	Isolated heart, application by perfusion with drug-seawater solution	57

* No declaration of unit.



Figures 3 and 4. Concentration-response-curves of the effect of acetylcholine (–●–) and nicotine (–□–) on the contraction amplitude (fig. 3) and frequency (fig. 4) of the isolated beating ventricle of *Sepia officinalis*. △ = acetylcholine response after treatment with cyproheptadine; ○ = acetylcholine response after treatment with d-tubocurarine (from Kling[57]).



Figures 5–8. The effect of noradrenaline, adrenaline, tyramine and serotonin on the isolated heart of *Sepia officinalis*. For further explanations see the text.

Table 4. The action of catecholamines and related components on the cephalopod heart

Species	Substance concentration	Effect	Preparation and mode of drug application	References
	Noradrenaline			
Sepia officinalis	5×10^{-9}–4×10^{-7} mol/l EC_{50}: 2.5×10^{-7} mol/l	Positive inotropic, positive chronotropic	Isolated heart (Straub cannula)	59
Octopus vulgaris	10^{-9}*	Positive inotropic, positive chronotropic	Isolated heart (Straub cannula)	25
Eledone cirrhosa	10^{-8}*	Positive inotropic, positive chronotropic	Perfusion of the isolated heart	74
	2×10^{-9}, 10^{-8}*	Positive inotropic, positive chronotropic	Perfusion of the isolated heart	26
	50 µg	Positive inotropic, positive chronotropic	Isolated heart	22
Octopus dofleini	300 µg/heart	Negative tonotropic, negative chronotropic, negative inotropic	In vivo, application through an implanted catheder into the efferent branchial vessel	47
	Octopamine			
Sepia officinalis	5×10^{-8}–3×10^{-6} mol/l EC_{50}: 3×10^{-7} mol/l	Positive inotropic, no chronotropic effect	Isolated heart (Straub cannula)	59
	Dopamine			
Sepia officinalis	10^{-8}–10^{-5} mol/l EC_{50}: 2×10^{-7} mol/l	Positive inotropic, negative chronotropic	Isolated heart (Straub cannula)	59
Eledone cirrhosa	5×10^{-6}*	Positive inotropic, no chronotropic effect	Perfusion of the isolated heart	26
	4×10^{-8}*	Inactive	Perfusion of the isolated heart	74
	Adrenaline			
Sepia officinalis	0.0005 mg	Positive inotropic, positive tonotropic,	Isolated heart	62
	0.005 mg	positive chronotropic		
Loligo pealii	10^{-9}–10^{-5}	Positive inotropic, positive chronotropic,	Isolated heart	9, 11
	10^{-4}*	systolic arrest		
Eledone cirrhosa	10^{-8}, 2×10^{-7}*	Positive inotropic, positive chronotropic	Perfusion of the isolated heart	74
	2×10^{-9}, 10^{-8}, 5×10^{-8}*	Positive inotropic, positive chronotropic	Perfusion of the isolated heart	26
Octopus dofleini	50–100 µg	Negative chronotropic	In vivo, application through an implanted catheder into the efferent branchial vessel	47
Octopus vulgaris	10^{-9}*	Positive inotropic, positive chronotropic	Straub cannula	25
	1–20 µg/heart	Positive inotropic, positive chronotropic	In vitro, isolated heart	91
	2–20 µg/kg	Negative chronotropic, negative inotropic	In vivo, application through an implanted catheder	
	100 µg/kg	Arrest		
Sepia officinalis	10^{-8}, 6×10^{-6} mol/l EC_{50}: 2×10^{-7} mol/l	Positive inotrope, no significant chronotropic responses	Isolated heart (Straub cannula)	59
Octopus vulgaris	2.0 µg	Positive chronotropic	In vitro	33
	8.0 µg	First negative inotropic then positive inotropic Increase of potential amplitude on the electrograms	Isolated perfused heart, application by injection into perfusion fluid	
	Tyramine			
Sepia officinalis	5×10^{-6}–7×10^{-5} mol/l EC_{50}: 5.5×10^{-5} mol/l	Positive inotropic, negative chronotropic	Isolated heart (Straub cannula)	59
	0.005–0.05 mg	Slightly positive inotropic, slightly negative chronotropic	Isolated heart	62
Loligo pealii	10^{-6}–10^{-3}*	Positive chronotropic	Isolated heart	9
Octopus macropus	5×10^{-6}–10^{-4}*	Positive inotropic	Isolated heart	14

Table 4 continued

Species	Substance concentration	Effect	Preparation and mode of drug application	References
Eledone cirrhosa	4×10^{-8}*	Inactive	Perfusion of the isolated heart	74
	5×10^{-6}*	Positive inotropic	Perfusion of the isolated heart	26
Octopus dofleini	100 µg	Negative inotropic, negative chronotropic	In vivo, application through an implanted catheder	47
Octopus vulgaris	10–100 µg/kg	Negative inotropic, negative chronotropic	In vivo, application through an implanted catheder	91
	20 µg/heart	Positive chronotropic, positive inotropic	In vitro, isolated heart	

* No declaration of unit.

area of their receptor arrangement that were hitherto unknown. We have no indications for the existence of a second excitatory ach-receptor in *Sepia* hearts as has been described for mytilids[41].

The reversibility of the ach-induced inhibition can be accounted for by the presence of high levels of acetylcholinesterase (EC 3.1.1.7.) and butyrylcholinesterase (EC 4.1.1.8.), which have been demonstrated in the central heart of *Sepia officinalis* by means of histochemical and cytochemical methods[56]. Biochemical methods have also identified cholinesterase in the cephalopod heart[12, 13, 42, 48, 67, 84].

It must be supposed that the extremely strong butyrylcholinesterase activity demonstrated in the inner myocardial layer strongly reduces the ach response[56]. This may be the reason for the relatively high ach concentration necessary for the inhibition of the *Sepia* heart, compared with that producing a response in vertebrate hearts.

The sensitivity of the cephalopod heart is generally the lowest of that in all molluscan classes; bivalve hearts even react to 10^{-12} mol/l ach, whereas gastropod hearts can be influenced in their action through 10^{-8} mol/l ach[37]. For *Octopus* on the other hand a threshold concentration of 10^{-7} mol/l is reported[93] and for the *Sepia* heart 6×10^{-7} mol/l ach[57]. It is known from vertebrate physiology that ach receptors of nerves and muscles are differently structured as regards their binding capacities; the differentiation between various vertebrate receptor types is characterized by their pharmacological properties, namely their sensitivity to the alkaloids nicotine and muscarine, and furthermore by their affinity to the blocking agents atropine, tetraethylammonium and d-tubocurarine. It may be concluded that such a distinct separation and classification of cholinergic receptors in invertebrates is impossible or only partially possible[28, 50, 66, 93].

Nicotine evokes an ach-mimicry on the heart of *Sepia officinalis:* concentrations of 6×10^{-9} up to 6×10^{-6} mol/l cause an inhibition of amplitude and frequency[57] (fig. 2). Thus the *Sepia* heart is more sensitive to nicotine than to ach itself (figs 3 and 4). This finding may again be explained by the high cholinesterase content of the *Sepia* myocardium[56]. Bearing this in mind we find a nicotinergic-like receptor in the *Sepia* heart. This finding is supported on the one hand by the fact that muscarine has no effect on the *Sepia* heart (table 2) and on the other hand that a significant blockade can only be produced by the snake venom α-bungarotoxin and by d-tubocurarine (table 3).

Additionally it must be noted that this blockade remains reversible; this indicates once again that there is no identity, only similarity between cephalopod and vertebrate cholinergic ach-cardioreceptors. A similar observation was made for neural ach receptors in *Aplysia*[52]. Furthermore it is shown that tetraethylammonium and atropine did not act as antagonists for the *Sepia* heart and that pilocarpine (acting predominantly with muscarine ach-receptors in vertebrates) has no effect[57]. With further respect to morphological[4, 53, 55, 81], histochemical[54, 56], cytochemical[56] and biochemical findings[12, 13, 42, 48, 67, 84] a cholinergic inhibitory innervation of the cephalopod heart may be ascertained.

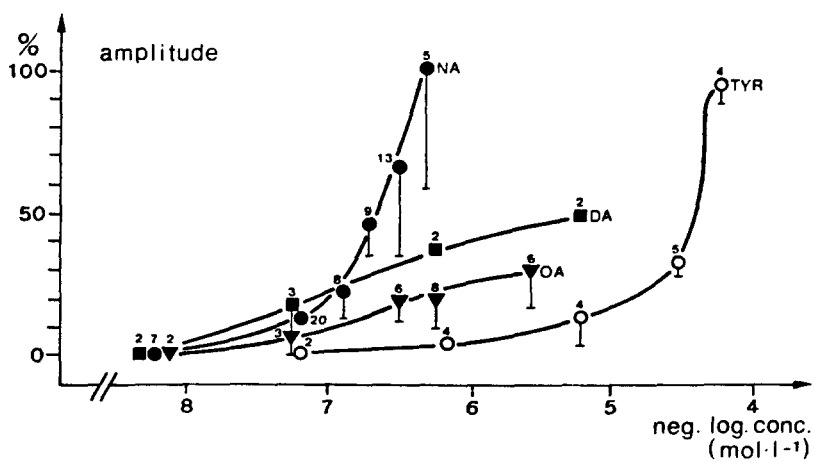


Figure 9. Concentration-response-curves for the effects of catecholamines and related compounds on the inotropic effect on the *Sepia* heart in vitro (NA = noradrenaline, OH = octopamine, TYP = tyramine, DA = dopamine) (from Kling and Schipp[59]).

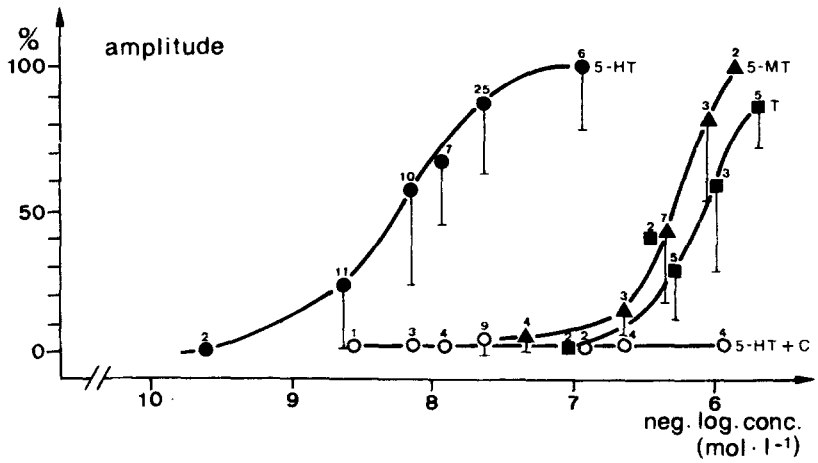


Figure 10. The action of indolamines on the concentration amplitude of the isolated beating *Sepia* heart 5HT = serotonin; 5MT = 5 methyl-tryptamine; T = tryptamine and the prevention of the 5HT effect cyproheptadine (from Kling and Schipp[59]).

Catecholamine compounds as candidates for neuronal transmission

Catecholamines are widely distributed in molluscs. But only a little quantitative description is devoted to the catecholamine content of tissues, especially of the heart. Only the cephalopod CNS has been investigated in more detail, with different methods, with respect to possible transmitter substances[82]. Pharmacologically we must distinguish between the 'triplets' noradrenaline, adrenaline and dopamine[87]. It seems that each of these amine systems has developed during molluscan phylogenesis; cardioexcitation can be demonstrated just as well as inhibitory effects. Thus manifold variants of the 'catecholamine-receptor principle' exist within the molluscan phylum[66]. Therefore the aminergic innervation must be considered separately for each molluscan species. Noradrenaline evokes a strong positive inotropic effect on the

Table 5. The action of indolamines on the cephalopod heart

Species	Substance concentration	Effect increase of potential amplitude on the electrograms	Preparation and mode of drug application	References
	Serotonin (5-HT)			
Sepia officinalis	10^{-9}–10^{-7} mol/l EC_{50}: 6×10^{-9} mol/l	Positive inotropic, no significant chronotropic effect	Isolated heart (Straub cannula)	59
	10^{-9}–10^{-8}, 10^{-6}*	Positive chronotropic, positive inotropic, positive tonotropic	Isolated heart, application by injection into perfusion fluid	29
Octopus vulgaris	4×10^{-8} mol/l	Positive inotropic, positive chronotropic	Isolated heart	14
Octopus dofleini	100 µg	Positive inotropic, slightly positive chronotropic	In vivo, application through an implanted catheder	47
Eledone cirrhosa	10^{-9}–10^{-8} g/ml 50 µg	Excitation Positive inotropic, positive chronotropic	Isolated heart	22, 23
Octopus vulgaris	1–20 µg/kg	Positive inotropic, positive chronotropic	In vivo, application through an implanted catheder	91
	1–3 µg/heart	Positive inotropic, positive chronotropic	In vitro, isolated heart	
Octopus vulgaris	0.5 µg 1 µg	Positive chronotropic First negative inotropic, then positive inotropic	In vitro, isolated perfused heart, application by injection into perfusion fluid	33
	Tryptamine			
Sepia officinalis	10^{-7}–2×10^{-6} mol/l EC_{50}: 6×10^{-7} mol/l	Positive inotropic, negative chronotropic	Isolated heart (Straub cannula)	59
Octopus vulgaris	10^{-6} mol/l	Positive inotropic, positive chronotropic	Isolated heart	14
	5-Methyl-Tryptamine			
Sepia officinalis	10^{-7}–1.5×10^{-6} mol/l EC_{50}: 5×10^{-7} mol/l	Positive inotropic, no chronotropic effect	Isolated heart (Straub cannula)	59

* No declaration of unit.

isolated *Sepia* heart (mean maximum effect: 117% increase) and causes a moderate increase of heart frequency (fig. 5).

These effects are dose-dependent. The threshold concentration for the NA-effects was 5×10^{-9} mol/l; the maximum stimulation was measured when applying 4×10^{-7} mol/l noradrenaline. A further increase of drug concentration evokes arrhythmia and systolic arrest. An EC_{50} value of 2.5×10^{-7} mol/l noradrenaline was evaluated[59] (fig. 9). These effects of noradrenaline on the *Sepia* heart can be blocked by phentolamine to a certain extent. The in vitro results contradict the in vivo effects of noradrenaline on the *Octopus* heart; cardioinhibition and a strong decrease of blood pressure were produced by injection of 20–100 µg/kg b.wt[91] resp. 300 µg/heart into the blood vessels of *Octopus*[47] (table 4). The intact circulatory system probably reacts to injected noradrenaline by showing strong peripheral vasodilation and thus entailing a blood pressure decrease; this vasodilation is not counterbalanced by the positive inotropic effect of noradrenaline on the myocardial musculatur.

In fluorescence histochemical studies, the intraaxonal fluorophores produced were characterized by means of cytophotometric analysis. The fluorphores were clearly

Table 6. The action of peptides on the cephalopod heart

Species	Substance concentration	Effect	Preparation and mode of drug application	References
	FRMF amide			
Octopus vulgaris	10–15 µmol/min	Positive inotropic, positive chronotropic (20%)	In vitro perfused heart	86
	+ naloxone (1–10 µmol/min)	Same as above		
Octopus vulgaris			In vivo ('free moving animal') injection into the:	89, 90
	4 µg (1450 g*)	Long-lasting positive inotropic	Branchial heart	
	4 µg (1450 g*)	Positive inotropic	Aorta	
	1 µg (827 g*)	Positive inotropic	Efferent branchial vessel	
	* = animal weight	all not chronotropic		
Sepia officinalis	9×10^{-8} mol/l	No effect	Isolated heart (Straub cannula)	This study
	9×10^{-8} mol/l–$3{,}4 \times 10^{-6}$ mol/l	Long-lasting positive inotropic, no chronotropic effect	In vitro perfused heart (perfusion with seawater drug solution)	
	EC_{50}: 7×10^{-7} mol/l			
	+ naloxone + phentolamine + cyproheptadin (equimolar: 10^{-5} mol/l)	Same as above		
	YGGFMRF amide			
Octopus vulgaris	10–15 pmol/min	Positive inotropic, positive chronotropic	In vitro perfused heart	86
Sepia officinalis	10^{-6} mol/l	Positive inotropic	In vitro perfused heart	
	YGGFMRF			
Octopus vulgaris	1 µmol/min	Positive inotropic, positive chronotropic	Isolated heart	86
Sepia officinalis	$< 10^{-5}$ mol/l	No effect	Isolated heart	
	10^{-5} mol/l	Positive inotropic (5–10%)		
Octopus vulgaris	met-enkephalinamide leu-enkephalinamide 10–15 pmol/min met-enkephalin leu-enkephalin 1 µmol/l	Positive inotropic, positive chronotropic	Isolated heart	86
Sepia officinalis	met-enkephalinamide leu-enkephalinamide met-enkephalin leu-enkephalin (10^{-9}–10^{-4} mol/l)	No effect	Isolated heart	This study
	Vasotocin			
	10^{-9}–10^{-6} mol/l	No effect		
	10^{-6} mol/l	Negative inotropic (− 20%), negative chronotropic (−10%)		
	Vasopressin			
	10^{-9}–10^{-5} mol/l	No effect		
	Cardiodilatin-28			
	$< 10^{-7}$ mol/l	No effect		
	10^{-7} mol/l	Negative chronotropic		

determined as catecholamine (NA, A DA) reaction products[56]. With respect to this and in consideration of the results obtained with biochemical investigations of the nervous tissue of cephalopods, where no adrenaline was found[49, 50, 51, 82], to regard noradrenaline as the natural transmitter of excitatory cardiac nerves seems to be the likely conclusion. Dopaminergic nerves have been discussed for the cephalopod CNS too, but this transmitter probably plays no role in cardioregulation because its pharmacological action is negligible[59].

Adrenaline stimulates the isolated *Sepia* heart too[59] (fig. 6), but this effect is interpreted as a noradrenaline mimicry because this transmitter does not exist in cephalopods, as mentioned above. Octopamine is found in high concentrations in the posterior salivary glands of *Octopus* vulgaris[24]. In the *Sepia* heart this amine shows only weak cardio-acceleration (fig. 9) and it can therefore not be regarded as putative transmitter in this species[59]. Within the molluscan phylum 'octopaminergic' nerves have only been described for *Aplysia*.

Complete noradrenaline mimicry with respect to the inotropic effect is produced by tyramine (figs 7 and 9), but only if this compound is applied in a 200-fold stronger concentration than noradrenaline. It is noteworthy that tyramine causes a negative chronotropic effect, in contrast to noradrenaline[59]. This finding cannot be interpreted in a satisfactory way. Such noradrenaline mimicry, with increased tyramine concentrations, has also been reported for *Loligo*[9], *Octopus*[74] and *Eledone*[26].

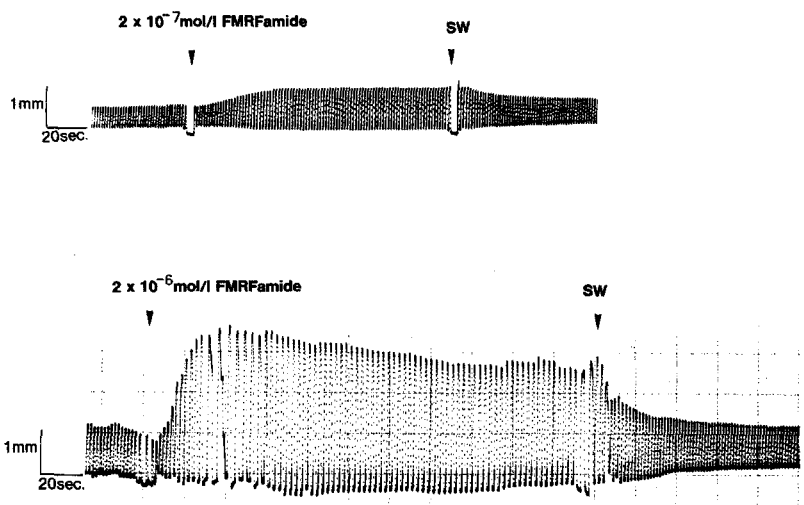
In experiments on the *Sepia* heart with common receptor antagonists only phentolamine proved to be slightly effective; the β-blocker propranolol as well as the dopamine receptor antagonist domperidone could not reduce the noradrenaline response[59]. A further characterization of myocardial catecholamine receptors by means of pharmacological application of the pure α-mimetic substance clonidine and of the β-mimetic agonist isoprenaline were not successful; neither of these substances evoked any considerable stimulation of the isolated *Sepia* heart at comparable drug concentrations[59]. Accordingly in *Sepia* heart muscle cells there is a catecholamine receptor which has the most sensitive reaction to noradrenaline and which can partly be blocked by phentolamine. We cannot speak about an α-receptor because the blockade is incomplete and the α-mimetic clonidine shows no response. Therefore the classification of cephalopod myocardial receptors by analogy to the determination of cardial receptors in vertebrates, as founded by Ahlquist[3], is not advisable.

What about serotonin (5-HT)?

Serotonin (5-HT) has been the most favored candidate for the excitatory transmitter in molluscan cardiac nerves for a long time[25, 28, 66, 79, 89]. The extremely high sensitivity of bivalve[60, 75] and gastropod hearts[20, 69] supports this hypothesis; the cephalopod central heart reacts to serotonin treatment with cardioexcitation too (table 6). In contrast to the catecholamine experiments, for serotonin in vivo and in vitro results correspond[91]. The *Sepia* heart shows a positive inotropic effect to 5-HT (max. response: 75% increase of amplitude), whereas no significant influence of heart frequency was measured[59] (fig. 8). An EC_{50} value of 6×10^{-9} mol/l serotonin for the inotropic effect indicates the high sensitivity of central heart muscle cells to this amine (fig. 10). On the other hand muscle cells of *Sepia* branchial hearts have no 5-HT receptor equipment; 10^{-7} to 2×10^{-5} mol/l serotonin did not show any effects[27]. With the 5-HT analogues 5 methyltryptamine and tryptamine similar effects on the *Sepia* heart could be triggered (fig. 10). Both related substances must be applied in 100-fold higher concentrations to mimic 5-HT effects[14, 59]. The 5-HT induced cardioexcitation can be antagonized by 3×10^{-6} mol/l cyproheptadine (fig. 10). In contrast methysergide shows agonistic properties and excites the heart; therefore this compound is not suitable for blocking experiments. An intrinsic effect for methysergide is not yet reported but the related compound lysergic acid (LSD) accelerates the venus heart[92].

Once again we conclude that the myocardial receptors in the cephalopod heart cannot be compared directly to the muscular or neural 5-HT receptors in vertebrates.

Furthermore, pharmacological attempts to differentiate between 5-HT_1- and 5-HT_2 receptors in the *Helix* heart



Figures 11 and 12. The concentration-dependent effect of FMRFamide on the isolated heart of *Sepia officinalis*.

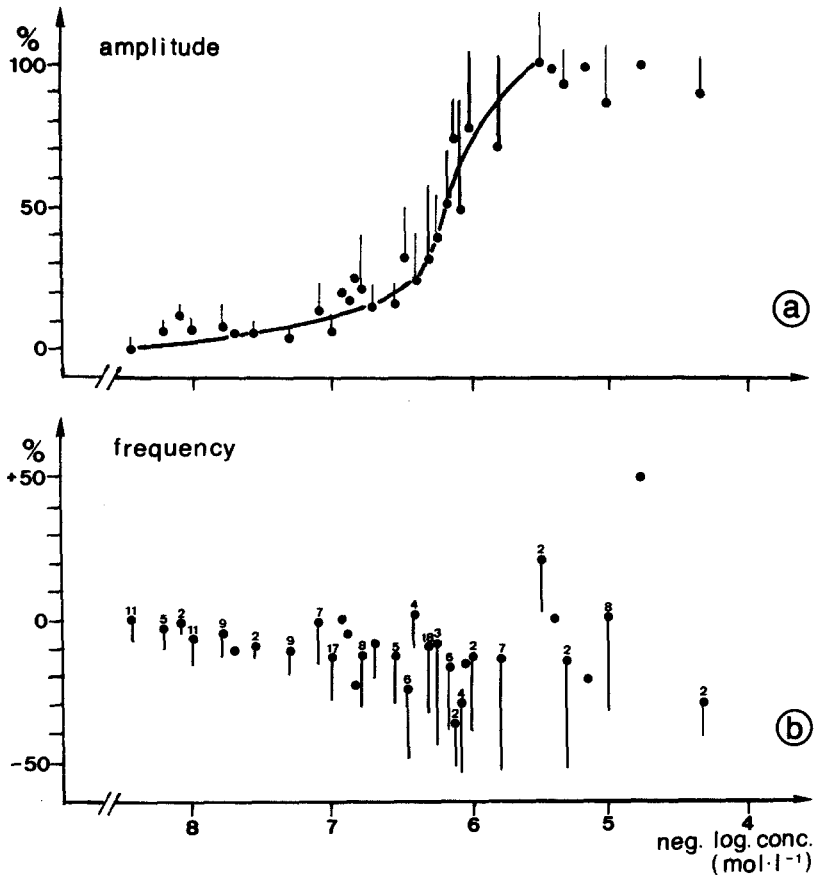


Figure 13. Concentration-response-curve of the effect of FMRFamide on the contraction amplitude (a) and frequency (b) of the isolated systemic heart of *Sepia officinalis*.

had no success and confirm the above-mentioned individual status of receptor properties in various molluscan species[20].

Cross-over experiments, where on the one hand the effect of serotonin was tested in the presence of phentolamine and on the other hand the effect of noradrenaline under cyproheptadine influence was measured, are of special interest. The fact that noradrenaline effects are not reduced by cyproheptadine pretreatment and, vice versa, the response of 5-HT cannot be impaired by phentolamine, leads to the conclusion that *two separate amine receptors* exist in the *Sepia* heart: one catecholamine receptor and a second one which is stimulated by serotonin. But what prevents us from postulating a serotoninergic innervation is the fact that up to now no serotonin could be found in cephalopod ventricles.

Whereas 5-HT was demonstrated by fluorescence histochemistry as well as biochemically in the cephalopod CNS[82,83], no indications for the presence of serotonin in cephalopod hearts were found with histochemical[56] and biochemical[51] methods. We can only speculate; either serotonin is released in vivo in other organs and tissues and reaches the central heart via the blood stream or, with externally applied 5-HT, we produce a mimetic stimulation of an unknown (peptidergic?) receptor.

Are there peptidergic receptors in the Sepia heart?

Morphological results suggest that in various species of cephalopods neurohormones are released into the bloodstream in the area of the anterior vena cava[5,6,70].

In earlier studies extracts of the 'neurosecretory system of the vena cava' (NSV-system) have been applied to isolated systemic hearts of different species of coleoids – the authors measured a cardioexcitatory response with the following properties[15–18,91].

– Compared to the short-lasting effects which are produced by known neurotransmitters like catecholamines, a characteristic long-lasting positive inotropic effect was caused in this case.

– Furthermore, the neurosecretion showed effective responses in low concentrations (ref. table 1).

– After application of high concentrations the systolic arrest was reversible.

– Effects on the heart activity of a similar nature were also induced after electrical stimulation of the NSV-system nerves.

The systemic heart of *Octopus bimaculaturs* and *Loligo pealii* were analyzed using biochemical methods to establish the content of cardioactive substances[1,2].

High concentrations of acetylcholine and four unidentified peaks (A, B, B° and C) in the chromatogram were found in the tissue extracts. Because the isolated heart of the bivalve *Mercenaria* shows a high sensitivity to known cardioactive transmitters the extracts were tested here in a bioassay. The authors considered the activity peaks B, B° and C to have the physiological role of cardioregulating substances, whereby the elution peak B° seems to be a catecholamine.

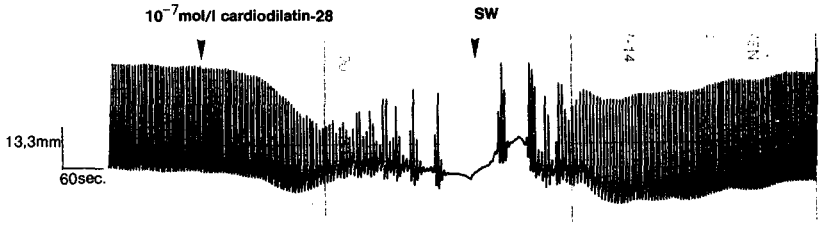


Figure 14. The effect of cardiodilatin-28 on the isolated perfused heart of *Sepia officinalis*. For further explanations see text.

After chromatographical research on the bivalve *Macrocallista nimbosa* Price and Greenberg found in ganglion extracts the activity peak C and, after further analysis, they could identify the tetrapeptide FMRFamide[76,77].
Investigations on different gastropods and bivalves revealed that this 'molluscan neuropeptide' can induce a cardioinhibition as well as cardioexcitation in different species[75].
The first indications of the presence of neuropeptides in the NSV-layer in octopods[71-73] gave rise to the question whether peptides – as in the vertebrates – influence in the mechanism of circulation in cephalopods and consequently whether there are also peptidergic receptors in the sarcolemma of the systemic and branchial hearts as well as in the blood vessels. Faced with this question we focussed in our group on the influence of some neuropeptides that occur in molluscs and vertebrates to investigate their activity on the isolated *Sepia* heart:
The molluscan neuropeptide FMRFamide induced a long-lasting increase of amplitude in the isolated systemic heart without significant modulation of frequency. We tested a total of 28 heart preparations and the following shows some typical actograms illustrating the effects of FMRFamide on the isolated systemic heart. Both in the isolated ventricle on the straub canulla and in the isolated perfused heart of *Sepia* $> 70^{-7}$ mol/l FMRFamide induced a strong positive inotropic effect which, after an overshooting of more or less 30 s, stabilized at a constant level and remained constant until the solution was changed – even over a period of several minutes.
The threshold concentration for the excitatory effect of FMRFamide on the isolated heart of *Sepia* was about 9×10^{-8} mol/l. After application of increasing peptide concentrations a continual increase of the amplitude was registered which, after reversal of application, was quickly reversible in every case. The EC_{50} is 7×10^{-7} mol/l FMRFamide.
An effect of about 100% (see dose-response-curve) corresponds to about a 3-fold increase of the amplitude and was induced by application of an 3×10^{-6} molar FMRFamide solution. The cardioexcitatory effect could not be diminished with phentolamine (ref. table 6).
Accordingly it seems that FMRFamide does not stimulate the α-like catecholamine receptor described above. Nor was the 5-HT blocker cyproheptadine able to block or to weaken the peptide-induced effects in the *Sepia* myocardium, when used in concentrations of 10^{-5} mol/l. Equivalent to this, 5-HT and FMRFamide may have an effect via two different types of receptors. This is in accordance with findings in bivalves:
Serotonin triggers a cardioexcitatory effect in the *Mercenaria* heart that can be blocked by methysergide[43,45]. This effect is mediated by an increase of the cAMP level[44,46]. However as the peptide-induced effect is not blockable with the serotonin antagonism either, Painter and Greenberg[75] presume that FMRFamide and 5-HT react by stimulation of two different receptor systems.
Furthermore opiate receptors that are blockable by naloxone cannot be presumed to be the sites of FMRFamide binding and effecting in the systemic heart of *Sepia*.
Voigt et al.[86] have proved in studies on the isolated *Octopus* heart, that even here a heart acceleration induced by XRF-NH_2 (peptideamides with the carboxy-terminal aminoacid sequence -Arg-Phe) cannot be evoked by a combination of the peptides with these opiate receptors.
When surveying the available findings we must conclude that in the systemic heart of *Sepia officinalis* FMRFamide acts by association with a new receptor type that is not identical with those receptors seen thus far. This gives rise to the question whether this tetrapeptideamide plays a part as a physiological agonist (not transmitter) in the cardioregulation of *Sepia officinalis* and in the other cephalopods. We must also ask in which organs (or tissues) peptides are synthesized and released.
As already explained FMRFamide shows a long-lasting positive inotropic effect in the systemic heart of *Sepia*

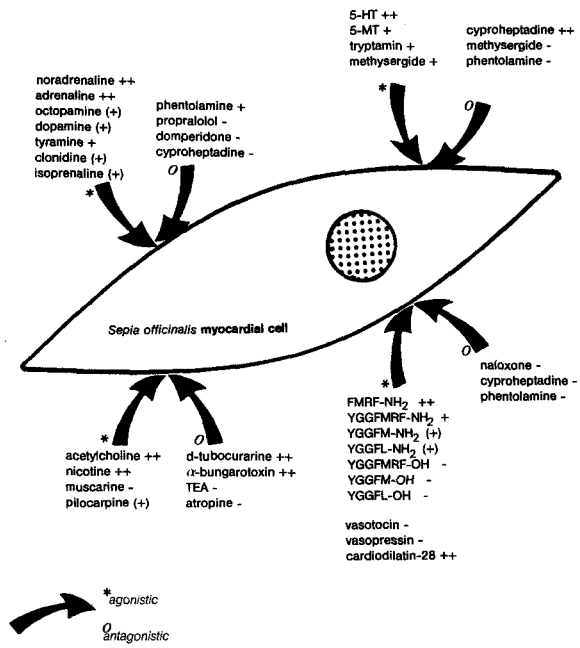


Figure 15. A schematic summary of various cardioactive substances; these effects are described in detail above. ++, strong effect; +, weak; (+), weaker; –, no effect.

that resembles the effect of NSV-extracts. Wells[89,90], who tested the effect of FMRFamide on the systemic heart of *Octopus vulgaris* in situ, therefore assumes that this peptide might be present in the NSV-system and is released by neurosecretion into the lumen of the vena cava in stress situations thus having a modulating effect on the central circulatory organs, especially on the systemic heart.

Our further research on the structure-activity relationship can exclude any similarity between desaminated analogues and FMRFamide as far as their influence as cardiomodulation substances in *Sepia officinalis* is concerned. Neither leu-, met-enkephalin nor met-enkephalin-Arg-Phe (YGGFMRF-OH) showed any effect. Met-enkephalin-Arg-Phe-amide (YGGFMRF-NH_2) did however induce a slightly positive inotropic effect in 10^{-6} molar concentration, and, when concentrations were increased (10^{-5} mol/l) met- and leu-enkephalinamide were also able to provoke a slight cardioacceleration. In conclusion we can therefore state that according to these results, FMRFamide has the highest affinity to the postulated peptidergic receptors.

In addition to the enkephalin-homologous peptides some other peptides belonging to the vasotocin-group were examined. Neither vasotocin nor vasopressin was able to modulate the heart activity (see table 6). Although material similar to vasopressin was localized in the NSV-system of *Octopus* in an immunocytological study[72], we can still maintain that these substances have no cardiotropic effects, at least not in *Sepia*.

The 'myoendocrine vertebrate peptide' cardiodilatin-28[30-32] induced a negative chronotropic effect in the isolated perfused systemic heart of *Sepia officinalis* (fig. 14). This peptide, belonging to the group of 'atrial natriuretic peptides' (ANF),[7,19] was initially only applied in sporadic tests so that no concentration-response-curve has yet been constructed. This cardiac peptide has been demonstrated phylogenetically by immunocytological methods in the atrial heart muscle tissue of organisms ranging from vertebrates to cyclostomes[80]. In *Helix pomatia* this peptide was also found in the heart, not in the myocytes but in terminal axons of the auricle[80]. In this gastropod the investigators were able to establish the presence of this peptide in the pericarya of the subesophageal ganglion and in visceral nerves.

In conclusion: The isolated systemic heart of *Sepia officinalis* can be stimulated in a dose-dependent way by the 'molluscan neuropeptide' FMRFamide. This response is neither due to stimulation of a catecholamine nor of an indolamine receptor and, in addition, this effect cannot be antagonized by naloxone. Thus we infer that peptidergic substances with the characteristics of FMRFamide participate in the hemodynamic and cardioregulation of the cephalopod circulation, even though their chemical structure, as well as the sites of synthesis and release (NSV-layer?) have not yet been ascertained. This will be the subject of attention in the future.

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The blood vessels of cephalopods. A comparative morphological and functional survey

by R. Schipp

Institut für Allgemeine und Spezielle Zoologie, Justus-Liebig-Universität, Stephanstr. 24, D-6300 Giessen (Federal Republic of Germany), and Station de Biologie Marine d'Arcachon, F-33120 Arcachon (France)

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)).