J Pharmacol Sci 112, 383 – 396 (2010)
Journal of Pharmacological Sciences

Critical Review

Some Embryological Aspects of Cholinergic Innervation in the Cardiovascular System — A Close Association With the Subintestinal Circulatory Channel

Tatsuro Shigei[1], Hiromichi Tsuru[2], Naohisa Ishikawa[3], and Koichi Yoshioka[4,*]

[1]*Department of Cell Pharmacology, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya 466-8550, Japan*
[2]*Department of Pharmacology, Toho University School of Medicine, Ohta-ku, Tokyo 143-8540, Japan*
[3]*Department of Pharmacology, Aichi Medical University, Nagakute-cho, Aichi-gun, Aichi 480-1195, Japan*
[4]*Graduate School of Sport System, Faculty of Physical Education, Kokushikan University, Tama, Tokyo 206-8515, Japan*

Received November 21, 2009; Accepted February 18, 2010

Abstract. A series of our studies on the dog venous system revealed that cholinergic excitatory innervation was localized in a group of veins: the portal, mesenteric, and hepatic veins and the middle segment of the inferior vena cava. Our studies on pharmacological responsiveness of dog veins also revealed that they could be divided into two groups: the visceral and somatic parts, and the cholinergic excitatory innervation localized to the visceral part. Considering these results and some relevant literature, a hypothesis is proposed on the classification of muscles of the cardiovascular system and some embryological aspects of the parasympathetic cholinergic innervation in the circulatory system are discussed. The embryonic circulatory system of vertebrates can be divided into two parts: somatic and visceral. The body of an embryo is regarded as a double tube and vessels of the visceral part and the heart belong to the inner tube. The muscle of these vessels and the heart are derived from visceral mesoderm, either the coelomic epithelium or mesenchymal cells, in common with muscle of the digestive tube; and thus the parasympathetic cholinergic nerves innervating the muscle of the digestive tube also distribute to these vessels and the heart. The heart and vascular muscles in the visceral part are structures developed early in the course of evolution in invertebrates. Their primary function is to propel the body fluid, and the chief structure containing them is the subintestinal circulatory channel (ventral aorta − heart − subintestinal vein). They exhibit spontaneous, rhythmic activity, showing characteristics of a single unit muscle, and receive parasympathetic cholinergic innervation. On the other hand, the vascular muscles in the somatic part are endothelium-associated muscles developed anew in the vertebrate; do not contract spontaneously, being classified as a multiunit muscle; and lack parasympathetic cholinergic innervation.

Keywords: embryology, cholinergic nerve, cardiac nerve, portal vein, inferior vena cava

Contents

*Corresponding author. yoshioka @kokushikan.ac.jp
Published online in J-STAGE on March 30, 2010 (in advance)
doi: 10.1254/jphs.09R04CR

1. Introduction

The subject of cholinergic innervation in the vertebrate circulatory system has been discussed from many viewpoints. The most prominent cholinergic influence on the circulatory system would be the inhibitory control on the heart through the vagus nerve. Vascular cholinergic innervation, on the other hand, is generally regarded as present at limited sites only, where the effect is either inhibitory or excitatory (1 – 4).

On cholinergic vasodilator mechanisms, Bell (5) reviewed the status of research up to 1991 and rated the extent to which the morphological or the pharmacological criteria support the existence of cholinergic vasodilator supplies to resistance vessels of several specific areas: skeletal muscle, uterus, penis, cerebral and coronary vessels, and exocrine glands. In some of the cholinergic vasodilator effects, the involvement of nitric oxide (NO) presumably released from endothelium has been subsequently reported (6 – 9). On the other hand, Toda and Okamura (10, 11) found that some of the non-cholinergic and non-adrenergic vasodilator responses induced by parasympathetic nerves were mediated by neurogenic NO. They established the presence of the nitrergic nerve in mammalian cerebral arteries and stressed the physiological role of nitrergic nerves in cerebral vasodilation, penile erection, and relaxation of sphincters in gastrointestinal and lower urinary tracts. A new version of the classical table of autonomic efferent nerves was proposed: The parasympathetic section should be divided into “cholinergic” and “nitrergic”.

On cholinergic vasodilator supplies, Bell (5) concluded that although the results of rating were variable, the functional importance was mostly questionable. On cholinergic vasoconstrictor mechanisms, on the other hand, Kalsner (4) reevaluated past reports on cholinergic effects on vasculature, and, based on some experimental evidence and evolutionary considerations, claimed that the direct action of neurogenically released acetylcholine (ACh) on the medial smooth muscle is predominantly constrictor even in mammals. Bell (5) also stated that the phenomenon of cholinergically mediated vasoconstriction had been confirmed only for certain intraabdominal vessels and appeared to be due to outgrowth of intestinal smooth muscle elements into the vasculature. The present review covers this problem and will show that the distribution of the smooth muscle characteristics and the cholinergic constrictor nerves follow certain morphogenetical principles, not mere outgrowth of muscle.

Previously, a series of our studies on the dog venous system revealed that cholinergic excitatory innervation was localized in a group of veins: the portal, mesenteric, and hepatic veins and the middle segment of the inferior vena cava (12 – 21). These veins are closely related to the digestive tube in their embryogenesis, and their smooth muscle probably derives from a common origin with visceral musculature. In the present review, some embryological aspects of the parasympathetic cholinergic innervation in the circulatory system are discussed, particularly focusing on the above findings as well as the innervation of the heart. In the basic pattern of the vertebrate circulatory system, a major circulatory channel, the subintestinal system, develops along the digestive tube ventromedially. The heart and the portal vein are components of the subintestinal system. In addition, the pattern of cardiac nerves well reflects the phylogeny of the autonomic nervous system.

2. Basic pattern of vertebrate circulation

The body of the vertebrate embryo is regarded as a double tube: the outer (somatic) portion and inner (visceral) portion. The former is the body wall and the latter is the digestive tube. The cardiovascular system belongs to both and may be divided in a similar way.

Figure 1 shows the basic pattern of the embryonic circulatory system (14, 22, 23). Early in the ontogenesis of vertebrates, a common pattern of cardiovascular system is formed, bilaterally, as a rule. It consists of three major channels: 1) the subintestinal system (ventral aorta − heart − subintestinal vein), 2) the supraintestinal system (dorsal aorta), and 3) the renal system (superior and inferior cardinal veins).

The subintestinal and the supraintestinal systems are connected by the branchial and the mesenteric vessels, and thus circulation of the digestive tube is first established. The cardinal veins (the renal system) then appear along the nephric ducts, as renal portal veins. [The pronephric structures vestigially extends on the cervical region even in mammals (24).] Intersegmental arteries perfuse the body wall (neural canal and myotomes in the figure), and the venous blood flows into the cardinal veins and returns to the sinus venosus via the ducts of Cuvier.

The subintestinal vein (the primary vein of the digestive tube) soon begins to retrogress from the anal end and is replaced by the secondary veins that appear supraintestinally along arteries. The mode of development of the primary and secondary veins in embryonic circulation of the digestive tube was discussed by Miki (22, 23).

The boundary between the somatic and the visceral parts of the circulatory system is shown by the dashed lines (Fig. 1: A and B). In this article, we do not discuss the renal system in the somatic part.

It should be noted that the basic pattern of circulation is formed of endothelial tubes, muscle being present only

in the heart. Medial smooth muscle appears later in the vascular wall during development (21).

3. Proposed hypothesis

In our previous review (21), we discussed the significance of the endothelium, medial smooth muscle, and sympathetic nerves in the vertebrate vascular system, from phylogenetical points of view. It seemed that in the course of evolution, all these components of the vascular wall appeared first in vertebrates. We presented a view that "the sympathetic nerve/medial smooth muscle system" may be regarded as a vascular neuroeffector mechanism that appeared and developed anew in the vertebrate circulation for systemic regulation of the "endothelium-lined closed vascular system". On the other hand, the parasympathetic nerves are thought to have appeared earlier already in invertebrates and have been distributed to the digestive tube and other viscera. We thus assume that the vertebrate vascular smooth muscles, which developed after the emergence of and in association with the endothelial tubular system, are not originally the target tissue of the parasympathetic nerves as a whole. This may account for the fact that whereas the sympathetic nerves distribute widely to the whole vascular system, the parasympathetic nerves are distributed in only limited sites.

Our proposed hypothesis on the cholinergic innervation is as follows (Fig. 1A): Muscles of the subintestinal system (ventral aorta − heart − subintestinal vein) are derived from the visceral (splanchnic) mesoderm, either the coelomic epithelium or mesenchymal cells derived from the epithelium, in common with muscle of the digestive tube. The cholinergic (parasympathetic) nerves distribute both to the muscle of the digestive tube and to that of the subintestinal system. The same principle is reflected in the adult circulation.

In the following sections, findings and information on which the hypothesis is based will be presented and discussed.

4. Brief summary of our results on dog veins

4.1. Dog veins: sites of cholinergic excitatory innervation

Table 1 summarizes the results of our studies on the dog venous system. Helical or longitudinal strips were prepared from sixteen sites and examined. The presence of cholinergic excitatory innervation was revealed as an atropine-sensitive component in the response to transmural electrical stimulation. Positive results were obtained only in four veins: the portal, mesenteric, and hepatic veins and the middle portion of the inferior vena cava (IVC) (segment B-C, see Fig. 2B). In addition, only these

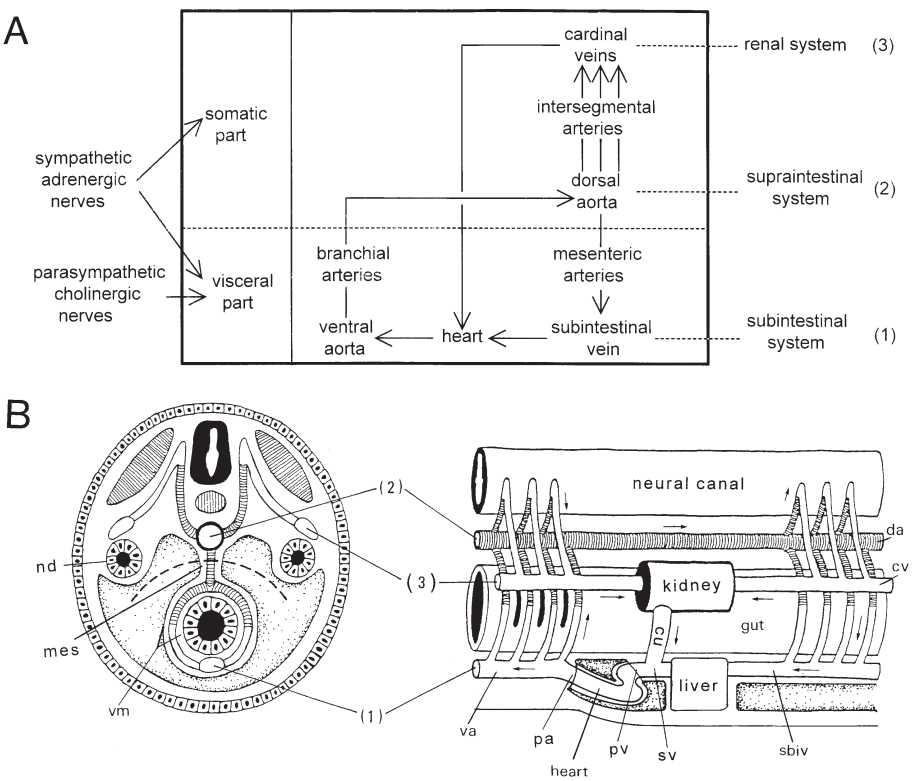

Fig. 1. Division of the cardiovascular system and distribution of the autonomic vasomotor nerves. An embryological view. A: Schematic diagram of the basic pattern of embryonic circulatory system of vertebrates. B: The cross section (left) and the lateral view (right) of a vertebrate embryo. In the cross section, a slightly more advanced stage of the mesenteric artery is drawn compared with the lateral view. Arrows show the direction of blood flow. cu: ductus Cuvieri, cv: cardinal vein, da: dorsal aorta, mes: mesentery, nd: nephric duct, pa: porta arteriosa, pv: porta venosa, sbiv: subintestinal vein, sv: sinus venosus, va: ventral aorta, vm: visceral mesoderm. (Reproduced from Ref. 23, with modification)

veins showed a leftward shift in concentration–response curves to ACh after anticholinesterase treatment and positive cholinesterase staining. These veins are rich in longitudinal muscle and exhibit a spontaneous rhythmic activity (Table 1d and Fig. 2C). It was also noted that the splenic vein did not belong to this group, although it is generally thought to be a portion of the portal venous system.

Figure 2A shows the responses of two veins to transmural electrical stimulation. In the portal vein, after α-adrenergic blockade with prazosin, the persistent small response was enhanced by an anticholinesterase (neostigmine), and was antagonized by atropine. In the middle portion (segment C) of IVC, the potentiating effect of anticholinesterase (physostigmine) was much greater (19).

In separate experiments with anesthetized dogs, the extrinsic nerve supply to the middle portion of IVC was studied to find out which nerve contains the cholinergic excitatory fibers. By means of a special intravascular cuff, the active tension development of the vascular wall could be recorded quantitatively, while preserving blood flow. A cholinergic constrictor response was recorded when the greater splanchnic nerve, but not the vagus nerve, was electrically stimulated (16). The same result was obtained in the portal vein, and it was occasionally, although slightly, observed in the pulmonary vein (25).

4.2. Differences in pharmacological responsiveness between two groups of veins

Table 1 also indicates that the portal and mesenteric veins and the middle segment of IVC behave distinctively in some other responses (e, f): These veins did not relax in response to ACh even in the presence of endothelium (26 – 28) and show very low sensitivity to the relaxant action of isoproterenol (29). The idea to classify veins into two groups —the visceral and somatic parts of veins— had been suggested by our earlier experiments. Distribution of responsiveness to bradykinin was studied in the dog venous system (30). It was revealed that veins

Table 1. Pharmacological characteristics of isolated canine veins: evidence for cholinergic excitatory innervation

	(a) Transmural stimulation	(b) AntiChE effect	(c) ChE stain	(d) Spont. contr.	(e) ED-relaxation to ACh	(f) Relaxation to Isp
Cephalic	−	−	−	−	+	+++
External jugular	−	−	−	−	+++	+++
Brachiocephalic	−	−	−	−	++	
Superior vena cava	−	−	−	−	+	
Azygos	−	−	−	−	+	+++
Pulmonary	−	−	−	−	+	+++
Inferior vena cava						
A	−	−	−	−	++	+++
B-C (H, L)	+	+	+	+	−	±
D	−	−	−	−	++	+++
Portal (H, L)	+	+	+	+	−	±
Mesenteric (H, L)	+	+	+	+	−	±
Hepatic (L)	+	+				
Splenic	−	−	−	−	++	+++
Renal	−	−	−	−	+	+++
Femoral	−	−	−	−	+	+++
Saphenous	−	−	−	−	+	+++

H: helical strips, L: longitudinal strips. (a): response to transmural electrical stimulation, potentiated by anticholinesterase treatment, and antagonized by atropine. (b): leftward shift of ACh concentration–response curves after anticholinesterase (antiChE) treatment. (c): cholinesterase (ChE) staining. (d): presence of spontaneous, rhythmic contraction. (e): endothelium-dependent relaxation to ACh. −: no relaxation, +: <20%, ++: 20% – 50%, +++: = 90%; % relaxation of methoxamine-induced contraction. (f): relaxation to isoproterenol; ±: <20%, +++: >80%; % relaxation of methoxamine-induced contraction. Data cited from the following references: (a), (b), and (c): Refs. 15, 31, and 19; (c) and (d): Ref. 17; (e): Ref. 26, and (f): Ref. 29.

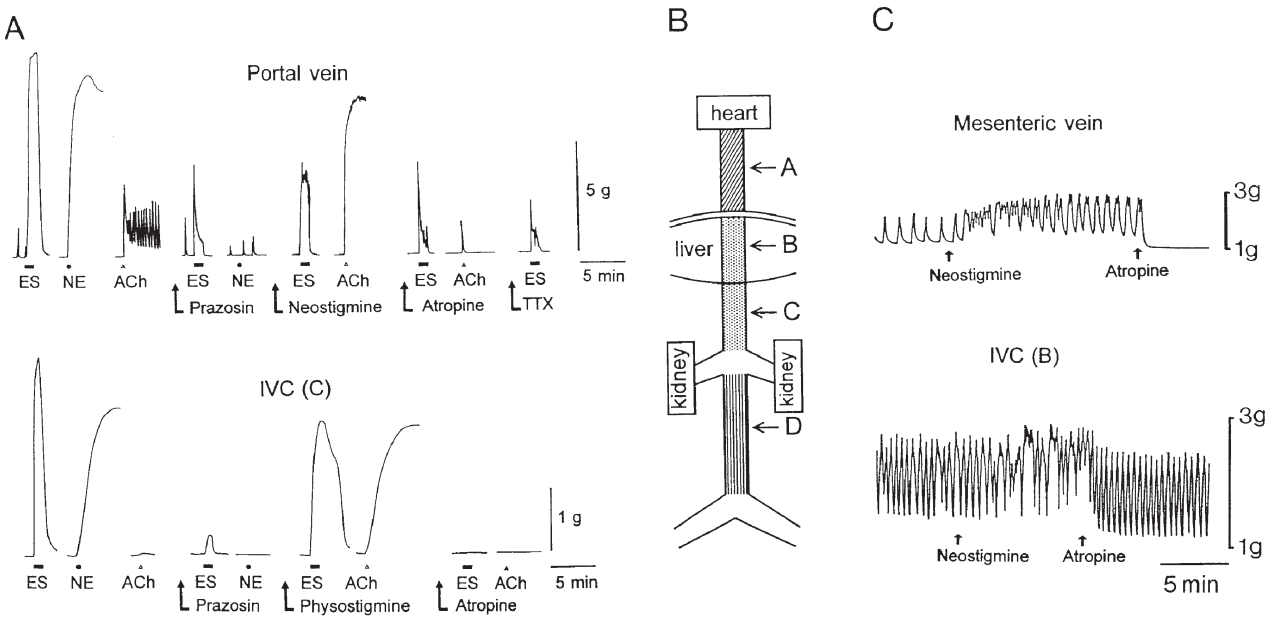

Fig. 2. Responses of canine veins of the visceral part and division of inferior vena cava. A: Effects of antagonistic agents and anticholinesterase treatment on responses of longitudinal strips of the portal vein and the segment C of the IVC. Control responses to electrical field stimulation (ES, 60 s at 30 Hz), norepinephrine (NE, 3 μM for portal vein and 10 μM for IVC), and ACh (0.3 μM) were first observed. Prazosin (1 μM) and neostigmine or physostigmine (1 μM) and then atropine (0.1 μM) were successively applied. In the portal vein, tetrodotoxin (TTX, 0.3 μM) was added after application of atropine. B: Division of canine IVC. C: The canine mesenteric vein and the segment B of IVC, longitudinal strips. Spontaneous activity and effects of neostigmine (1 μM for mesenteric vein and 0.1 μM for IVC) and atropine (40 nM).

were divided into two groups: high or low responsive to bradykinin. The distribution was correlated with embryogenesis of the system. In another experiment, sensitivities of veins to norepinephrine (NE), serotonin (5-HT), and histamine were examined (13, 31). Here, the point of the latter experiment will be presented briefly.

Figure 3 schematically indicates the distribution of those veins that are highly sensitive to NE (hatched portions), 5-HT (stippled portions), and histamine (solid portions). With helical strips prepared from fifteen sites of veins, pD_2 values ($-\log EC_{50}$, M) for the agents were obtained from the respective concentration–response curves. A histogram was drawn for each agent by stacking the overlapping ranges (mean ± S.E.M.) of the pD_2 values of all fifteen veins. The obtained histogram proved to consist of two separate components: a high and a low sensitive group of veins. In the figure, veins that showed higher sensitivities are marked.

It can be seen that, as a whole, the veins of the somatic part (outer tube) are highly sensitive to NE and 5-HT, while the veins of the visceral part (inner tube) (the portal, mesenteric, and hepatic veins, the middle portion of IVC as well as the pulmonary vein) are highly sensitive solely to histamine. The hepatic vein has the same characteristic as these veins (18), as is also indicated in the

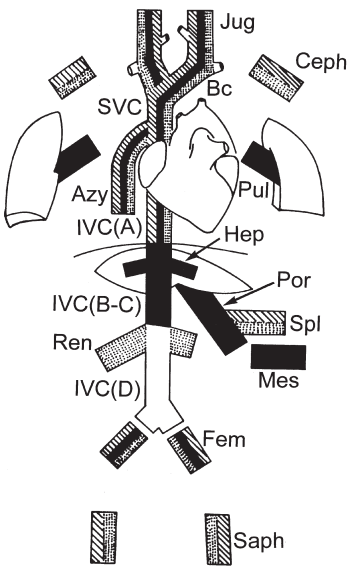

Fig. 3. Schematic diagram showing distribution of sensitivities to vasoactive substances in the dog venous system. Marked veins are highly sensitive to norepinephrine (hatched), serotonin (stippled), and histamine (solid). Jug: external jugular vein, Ceph: cephalic vein; Bc: brachiocephalic vein; SVC: superior vena cava; Azy: azygos vein; Pul: pulmonary vein; Hep: hepatic vein; Por: portal vein; Mes: mesenteric vein; Spl: splenic vein; Ren: renal vein; Fem: femoral vein; Saph: lateral saphenous vein; IVC (A), IVC (B-C), and IVC (D): segments A, B-C, and D of the inferior vena cava, respectively.

figure.

In this experiment, we made no pretreatment of the preparation. Neither neural uptake nor α- or β-adrenoceptor was blocked before application of each agent. Examination of data by normalizing all pD_2 values, taking the calculated experimental error as unit, showed the following: For each agent, 1) the means of high- and low-sensitive groups differ significantly, and 2) the magnitude of variation in sensitivity (SD of normalized pD_2 values) in each group, where several sites of veins are included, did not exceed that of an individual veins. Now we think the result is reflecting a regional distribution of "in vivo" sensitivities of smooth muscle in the dog venous system to these endogenous amines. Although interpretation of such an experiment is never simple, the results provide a useful overview of regional heterogeneity in the whole venous system.

4.3. The inferior vena cava, an embryologically mosaic vessel

The IVC is an embryologically mosaic vessel (24, 32, 33). In the canine IVC, we found that there exist segmental differences in vascular characteristics that well correlate to the embryogenetic distinction (12, 13, 15, 34). As schematically shown in Fig. 2B, these segments were termed A (supradiaphragm), B-C (intrahepatic and that between liver and renal veins), and D (infrarenal). These segments are known to be derived from distinct origins of the embryonic venous system.

The middle segment (B-C) resembles the portal and mesenteric veins, with respect to the presence of a thick layer of longitudinal muscle, spontaneous, rhythmic contractions, and cholinergic excitatory innervation as well as mode of pharmacological responsiveness. Such features are entirely different either from segment A or D. The wall of segment B-C receives its blood supply via the right phrenicoabdominal artery. When a dye was injected into the artery, only segment B-C was stained. [Segment B-C is a continuous structure, the pacemaker for spontaneous contractions being located in segment B. In our early report (12), we described that both segments had pace maker activity, but later it was shown that only segment B shows the activity.] Figure 2C shows the rhythmic contraction and the response to neostigmine and atropine recorded in longitudinal strips cut from the mesenteric vein and the segment B of IVC.

The similarity of segment B-C of IVC to the portal and mesenteric veins is thought to come from their mode of development. In an amphibian embryo, K. Takaoka (35) (directed by Dr. R. Ura) found that the primordium of the middle portion of IVC was formed within the right accessory mesohepaticum between the efferent vein of the liver and, caudally, the right subcardinal vein (Fig. 4). Such development of the middle portion (segment B-C in the dog) between a double sheet of peritoneal, serous membrane (mesohepaticum) may account for its similarity to the portal and mesenteric veins that likewise develop within the mesentery. Smooth muscle cells of these vessels possibly originate from the serous membrane (R. Ura, personal communication). Thus we regard the middle portion (B-C) of IVC as one of the vessels of the visceral part. A similar mode of development of this portion was also stated by Romer and Parsons (33).

Species difference is remarkable regarding the relative size of each segment of IVC. Segment A is extremely short in human IVC. Segment B-C is hardly recognizable in the rabbit. In the domestic foul, however, the presence of cholinergic excitatory innervation was reported by

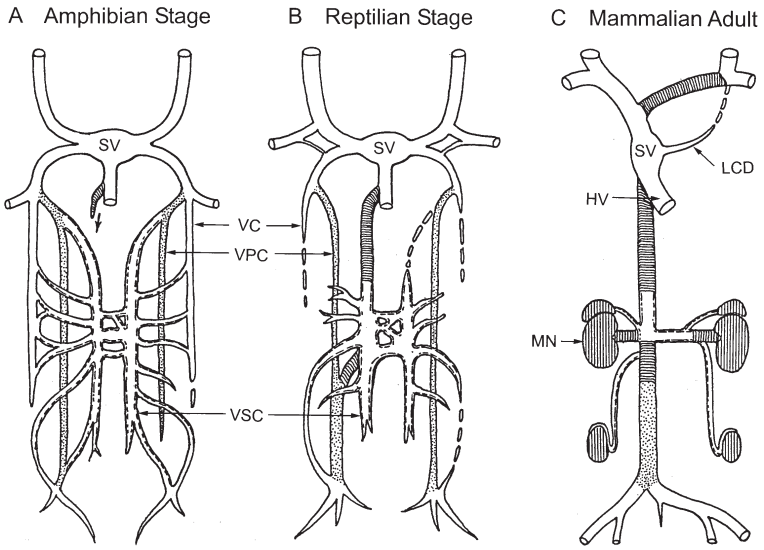

Fig. 4. Development of the IVC of mammals, showing the embryological processes reflecting the phylogenesis. I: amphibian stage, II: reptilian stage, III: mammalian adult type. SV: sinus venosus, VC: cardinal vein, VSC: subcardinal vein, VPC: paracardinal vein, MN: metanephros, LCD: left Cuverian duct, HV: hepatic vein. (Reproduced, by permission, from Ref. 22, with modification)

Bennett and Malmfors (36) (Table 2). It was in the portion between the liver and the adrenal glands, with a well-developed longitudinal muscle coat. Spontaneous rhythmic activity was occasionally seen. Therefore, it is highly probable that the observed segment of IVC in the domestic foul is embryologically homologous with segment B-C of the dog.

4.4. The splenic vein and spleen

The splenic vein is generally regarded as a portion of the portal venous system. However, we found that vascular characteristics of the splenic vein were markedly different from those of the portal vein in several aspects: pharmacological responsiveness to vasoactive agents (potentiation of ACh-induced contraction by neostigmine, relaxation response to isoproterenol, and contractile response to 5-HT); absence of longitudinal muscle layer; and no spontaneous contraction; presence of semilunar valves, which are known to be absent in the portal and mesenteric veins (13) (Table 1 and Fig. 5B). [In this regard, many microvalves were found by Takeshige (37) in the human mesenteric venules.] In contrast, the pharmacological responsiveness of strips cut from the splenic capsule and trabeculae, which contain abundant smooth muscle tissue, was rather similar to the portal vein (38) (not shown in the figure). Thus the splenic vein can be regarded as a distinct segment of vessel inserted between the portal vein and the spleen.

The distinctness of the splenic vein may be explained as a reflection of its unique embryogenesis, which was revealed by Miki (39, 23) (Fig. 5A). In his study with chick embryos, Miki clarified the following sequence of vascularization of the spleen: The spleen primordium arises on the dorsal wall (the greater curvature) of the stomach. It is penetrated by the stomach portion of the

Table 2. Vessels of the visceral part that show cholinergically induced excitatory responses

Vessels	Species	Reference
a) Gill circulation	many fish	e.g., 43
b)* Ventral aorta	eel, trout	64
c) Pulmonary vasculature	toad	69
d)* Pulmonary artery	lizard, tortoise	44
e)* Anterior mesenteric artery	domestic fowl	45
f)* Inferior vena cava	domestic fowl	6
g)* Mesenteric vein	sheep	70
h)* Mesenteric vein	cattle	71
i)* Inferior vena cava (middle segment)	dog	15, 16
j)* Portal and mesenteric veins	dog	19, 25
k) Hepatic vein	dog	18
l)* Portal vein	rabbit	72
m) Coronary artery	cattle	73
n)* Coronary artery	human	46

Atropine-sensitive responses were evoked by stimulation of vagosympathetic or vagus nerve (a, d), the intracranial roots of the vagus (c), transmural stimulation (b, e – n), stimulation of the periarterial nerves of the accompanying artery (g), of the greater splanchnic nerve (i, j). Potentiation of the responses by anticholinesterase treatment was clear in e, i – l, and n. Hexamethonium blocked the response in e, but was ineffective in g and i. *: presence of spontaneous, rhythmic contraction.

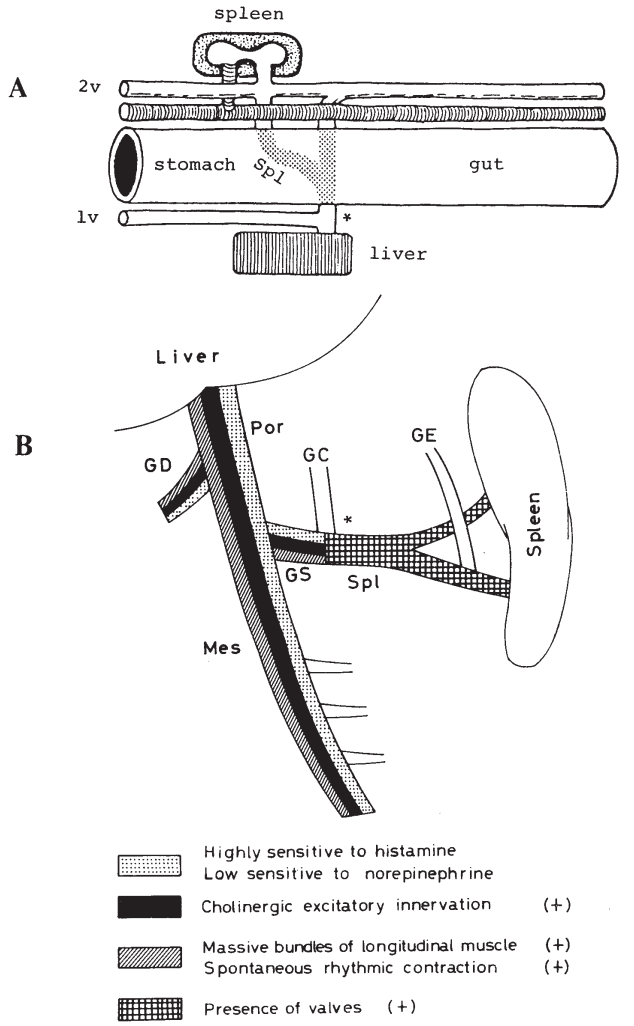

Fig. 5. A: Scheme showing the relation of the splenic vein (Spl) to the primary vein (1v) and the secondary vein (2v) in the vascularization of digestive tube. B: Scheme showing vascular characteristics in the dog portal venous system. Por: portal vein, Mes: mesenteric vein, GD: gastroduodenal vein, GC: gastrocoronary vein GE: gastroepiploic vein, GS: gastrosplenic vein. 1v, 2v and Spl in A correspond to GC, GE and Spl in B, respectively. The artery in A is a longitudinal anastomosis passing along the dorsal side of the digestive tube (23). (Reproduced from Ref. 13)

secondary vein, which flows into the portal vein, thus serving as an efferent vein of the spleen. At a later stage, however, a shunt is formed between the spleen and the portal vein as a newly formed efferent vein in association with separation of the spleen from the gastric wall. This shunt represents the splenic vein of the adult. Such a mode of development is thought to be the basis of the distinctiveness of the splenic vein among the portal venous system.

With respect to the similarities between smooth muscle of the splenic capsule-trabeculae and that of the portal vein, it is interesting that a spontaneous rhythmic activity has been demonstrated in the spleen of several mammalian species (40, 41). Although we have not so far demonstrated in the canine splenic capsule, a cholinergic innervation has been reported to be present in the spleen of the cod (42). Since the spleen primordium develops in the wall of the stomach, it is probable that the visceral coelomic epithelium, which covers it, also provides the spleen with smooth muscle.

5. Vessels of the visceral part showing cholinergic excitatory responses

Table 2 shows those blood vessels of the visceral part in which a cholinergic excitatory response to electrical nerve stimulation was demonstrated in either in vitro or in vivo experiments. The facts that cholinergically evoked responses have been detected in various sites of vessels of the visceral part and that many of them are spontaneously active (asterisks in Table 2) are consistent with our hypothesis.

As regards to the functional significance, the degree of contraction is not uniform, according to preparations and species. Some physiological role was postulated, for example, in the gill circulation of fish (43), the pulmonary artery of lizard (44), the anterior mesenteric artery of fowl (45), or the human coronary artery (46). In dog veins, the cholinergically evoked contraction of veins of the digestive tube is generally so small, compared with the adrenergically evoked contraction, that any physiological significance of the former in the neural vascular regulation is not clear (Fig. 2A).

6. Basic pattern of the cardiac nerves

In order to understand the embryological nature of cholinergic innervation to the heart, it is helpful to compare it with the innervation of the branchial nerves to the branchial muscle.

6.1. The branchial muscle and the heart

Romer and Parsons (33) presented the following scheme as "the most natural classification of muscles":

Somatic musculature { Axial { Trunk and tail; Eye ball } ; Appendicular (limbs*) }

Visceral musculature { Branchial (striated); Smooth (gut, and the like) }

*added by the present authors.

The somatic musculature forms the muscles of "the outer tube" of the body, and is universally striated. It is derived from myotomes and is innervated by somatic motor nerves. In "the inner tube", the visceral musculature, which is connected mainly with the gut tube, is composed of both striated and smooth muscles, which are located in the anterior (branchial) and posterior (postbranchial) portions of the digestive tube, respectively. In the head and pharyngeal region, functions of eating and breathing necessitated the development of striated muscle. These muscles, both striated and smooth, are derived from visceral mesoderm, and innervated by visceral motor nerves.

In the subintestinal channel, muscle differentiation, similar in the digestive tube, is observed: the heart is striated and a visceral muscle and thus may be regarded as the counterpart of the branchial muscle. Figure 6A illustrates the segmental innervation of the branchial muscle by the branchial nerves: V, VII, IX, and X of the cranial nerves and innervation of the sinus venosus by a branch of the vagus nerve. According to Miki (22, 47), circulation of the stage of the basic pattern is maintained by three portal veins: 1) the hepatic portal vein, 2) the renal portal vein, and 3) the heart, which can be regarded as the branchial portal vein.

In the course of the evolution of vertebrates, the transition from gill-breathing to lung-breathing associated with landing resulted in a large modification of the branchial muscle and the heart and additional great vessels (Fig. 6B). The branchial muscle differentiates, in mammals, to the muscles of pharyngeal, facial, and neck region (muscles for mastication, facial expression, and swallowing, etc.). The innervation to these muscles is well retained, and it is not difficult to trace the changes following the course of evolution (33).

6.2. Basic pattern of the cardiac nerves

The cardiac innervation —the vagus nerve, as well as the sympathetic nerves' supply— has greatly altered in its path and distribution in phylogeny, and the cardiac nerve plexus of mammals is extremely complicated and difficult to be analyzed.

The vertebrate heart receives cholinergic innervation,

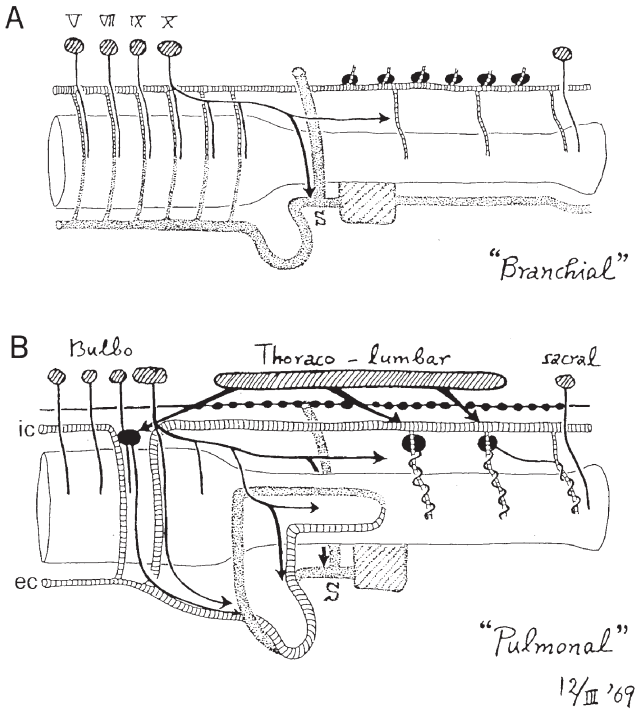

Fig. 6. Scheme showing major circulatory channels and distribution of autonomic nerves in vertebrates with a) branchial respiration and b) pulmonary respiration. The vessels containing arterial blood are hatched; those containing venous blood are shadowed. In a), nerves reach the heart only via the porta venosa. In b), after transformation of branchial arch arteries, nerves run along large arteries and pass the porta arteriosa (cf. Fig. 1B). V, VII, IX, X: trigeminal, facial, glossopharyngeal, and vagus nerves, respectively. Paravertebral and prevertebral sympathetic ganglia are shown in black circles. ic: internal carotid artery, ec: external carotid artery, S: sinus venosus. [This figure was drawn by S. Miki in 1969, based on his study of cardiac nerves (50) and the opinion of J. Botár (74) on phylogeny of the autonomic nervous system. It was not published during his lifetime.] (Reproduced by permission from Ref. 47, with modification)

generally inhibitory, via the vagus nerve. Exceptionally, in the lamprey heart, the vagal innervation is mainly excitatory, acting via nicotinic cholinoceptors (48, 49).

Hirakow and Miki (50) made a comparative study in order to clarify the basic pattern of the vertebrate cardiac nerves from a phylogenetic point of view (Fig. 7). Hearts were dissected in the shark, the turtle, and a human cadaver whose heart displayed a primitive pattern of innervation.

This study was based on an important observation of Ogawa (51). During the study of some cetacean cardiac nerves, he noticed a remarkable difference of the distribution pattern of the plexus between "porta arteriosa" (arterial gate) and "porta venosa" (venous gate). He found that the nerves at the porta arteriosa appeared to innervate the heart contralaterally, whereas those at the porta venosa did so ipsilaterally.

Before describing the results, it must be noted that the feature of cardiac innervation is related to the location of the heart within the animal body (Fig. 1B). The vertebrate heart develops just caudal to the gills or pharynx and cephalic to the point where the sinus venosus receives all venous blood from the body wall and the digestive tube via the ducts of Cuvier and the hepatic vein. After perforation of the dorsal mesocardium, the heart is completely surrounded by the pericardial cavity except for the cephalic or bulbus end (porta arteriosa) and the caudal or sinus end (porta venosa). Therefore, nerves can only reach the heart either through the porta arteriosa or the porta venosa (50, 52).

In the shark, the heart was innervated by the last branch of branchial rami of the vagus nerve only through the porta venosa, ipsilaterally. The nerves ran along the ducts of Cuvier to reach the sinus venosus (Figs. 7, left and

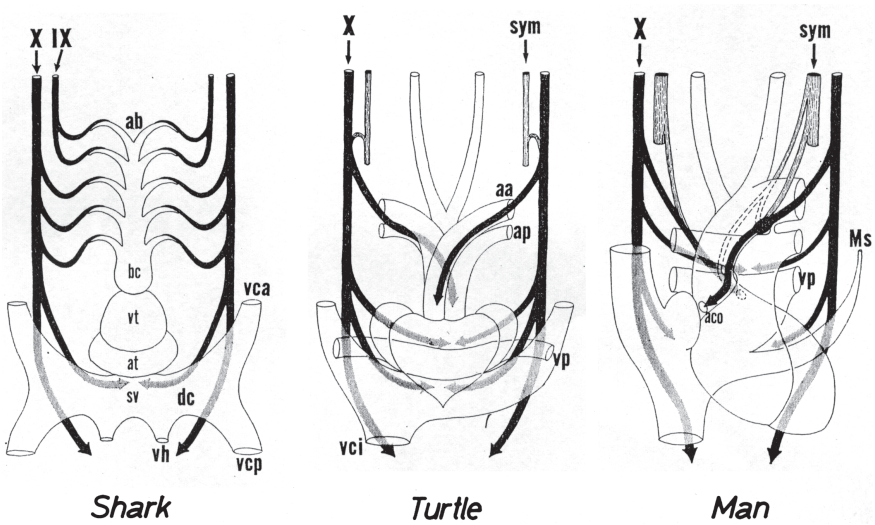

Fig. 7. Schematic drawings of the cardiac nerves of the shark, turtle, and the man. IX: glossopharyngeal nerve, X: vagus nerve, aa: arch of aorta, ab: branchial arch arteries, aco: coronary artery, ap: pulmonary artery, at: atrium, bc: bulbus cordis, dc: ductus Cuvieri, Ms: Marshall's vein (oblique vein of left atrium, remnant of the left Cuvierian duct), sv: sinus venosus, sym: sympathetic trunk, vca: anterior cardinal vein, vci: inferior vena cava, vcp: posterior cardinal vein, vh: hepatic vein, vp: pulmonary vein, vt: ventricle. (Reproduced, by permission from Ref. 47) For realistic drawings, see Refs 50 and 53.

6A). At the porta arteriosa, no nerve could be found. It appears that the row of gill-arches does not permit the penetration of any nervous components into the bulbus cordis. No sympathetic element was observed.

In the turtle (Fig. 7 middle), a new set of cardiac nerves appeared at the porta arteriosa, where nerves innervated the ventricle contralaterally, reflecting the modifications that take place in the outflow region during development (division of the bulbus into the aorta and pulmonary trunk, formation of the aortic arch, torsion of the bulbus, and so on.). At the porta venosa, on the other hand, the heart remains in its original state, except for the merging of the pulmonary veins, and an ipsilateral pattern of nerves like the shark was preserved. An anastomosis of the sympathetic trunk with the vagus was observed. This feature of cardiac innervation in the turtle is thought to be the basic pattern in terrestrial lung-breathing vertebrates. In the human case, the same pattern of innervation could be recognized (Fig. 7, right), although it was exceedingly modified by extreme asymmetry and by the sympathetic component markedly dominating the vagus. A lateral view of such innervation is illustrated in Fig. 6. The sequence of appearance of cardiac nerves —the vagus, the sympathetic nerves, and marked development of the latter— well reflects the phylogeny of the autonomic nervous system.

In addition, Hirakow (53) pointed out that the branch of the right vagus nerve went to the sinus node, while that of the left vagus nerve went to the AV node. This coincides with the physiological observation that electrical stimulation of the right or left vagus nerve of the dog causes inhibition of sinus pacing or AV conduction, respectively (54 – 56).

7. Some embryological aspects of cholinergic innervation in the cardiovascular system

7.1. Separation of sites of development of the circulatory system

In embryogenesis of the vertebrate circulatory system, endothelial network first appears on both sides of the wall of the digestive tube (the endodermal tube). The dorsal and ventral borders of the network become major channels. These initially develop bilaterally, but soon unite into single channels. Thus, two longitudinal channels that penetrate the body axis develop along the dorsal and ventral walls of the digestive tube: the dorsal aorta and the subintestinal channel (ventral aorta − heart − subintestinal vein). From the dorsal aorta, arterial branches arise in a segmental and paired manner (Fig. 1B, right). Then, upon formation of the mesentery, these arterial branches become unpaired, decrease in number, and give rise to visceral arteries (57) (Fig. 1B, left). Now separation of the outer (somatic) and inner (visceral) tubes of the body is evident. Sites of development of vasculature are also separated. At the developmental stage showing the basic pattern of circulation, the whole vascular system is composed of endothelial tubes, muscle being present only in the heart. Successive rearrangements of channels follow, resulting ultimately in the adult form of circulation. During such development, muscle appears in the vascular wall, site to site.

7.2. Classification of muscles in the cardiovascular system

It has been generally accepted that the origin of involuntary muscles, including enteric muscles, heart, and vascular muscles, is mesenchyme of either mesoderm or ectoderm origin in the outer tube of the body and is visceral mesoderm in the inner tube (24, 32, 58). Thus, concerning vascular muscles in the somatic part, medial smooth muscle of both arterial and venous walls are derived from surrounding local mesenchyme, which is of mesoderm origin. Muscles of the large arteries derived from the branchial arches (aortic arch, pulmonary arteries, brachiocephalic trunks, and common carotid arteries) are known to arise from neural crest cells, which are of ectoderm origin (59). In the visceral part, the origins of arterial smooth muscles are various among species. The visceral arteries arise from the aorta and distribute to gut wall through the mesentery. Their smooth muscles seem either to be extensions of those of the aorta or to derive from the serous membrane of the mesentery (visceral mesoderm). The dog mesenteric artery appears to be an example of the former because it shows no spontaneous activity and no cholinergic innervation (our unpublished observation); the foul mesenteric artery shows spontaneous activity and cholinergic innervation (45), suggesting that its muscle is of visceral mesoderm origin. The smooth muscles of veins (originally the subintestinal system) are derived from the visceral mesoderm, either the coelomic epithelium or mesenchymal cells derived form the epithelium, in common with muscle of the digestive tube. The above statement may be summarized as follows:

		muscle origin
somatic part	arteries	somatic mesenchyme or neural crest
	veins	(“vascular” muscle)
visceral part	arteries	species-dependent
	veins	visceral mesoderm
		(“visceral” muscle)
	heart (striated)	visceral coelomic epithelium

Our study covers the veins of both somatic and visceral parts, and the results strongly suggest the distinction between the two groups of veins.

In this regard, Wake found a definitely uneven distribution of retinol between two kinds of mesenchymal cells in the body of lamprey, *Lampetra japonica*. Almost all fibroblast-like cells in the splanchnic organs, including the liver, digestive canal, pancreas, kidney, gonads, heart, and gill, contain large amount of retinol. In contrast, retinol-storing cells are sparse or absent in somatic tissues such as dermis, subcutaneous tissues, notochord and nervous system, and skeletal muscle (60).

Hirakow (61) made a comparative study on the embryonic tubular heart of newt, heart of tunicate, and pulsatile, subintestinal vessel of amphioxus by means of electron microscopy. Based on the results, and some relevant reports, he stated that in the chordates (prochordates and vertebrates), the primary tissue bounding the circulatory channels and propelling the body fluid is universally coelomic epithelium itself containing myofilaments. The endothelium is not essential, arising only in the vertebrate. We also discussed the latter issue in our previous review (21). Furthermore, as Hirakow pointed out, in some invertebrates such as an annelid, a homologous coelomic myoepithelium was observed in the wall of dorsal longitudinal vessel, which is pulsatile and drives blood (62). Some phylogenetic relationship of these structures with the origin of the vertebrate heart was suggested.

Considering these, we will try to propose a possible scheme of the classification of muscles in the cardiovascular system and its biological significance, in particular, the presence of cholinergic innervation, as follows: The heart and vascular muscles in the visceral part are structures developed early in the course of evolution in invertebrates. Their primary function is to propel the blood and the chief structure containing them is the subintestinal circulatory channel. They exhibit spontaneous rhythmic activity, showing characteristics of unitary (single unit) muscle (56, 63) and receive parasympathetic cholinergic innervation. On the other hand, the vascular muscles in the somatic part are endothelium-associated muscles developed anew in the vertebrate (21). They do not contract spontaneously, being classified as a multiunit muscle, and lack parasympathetic cholinergic innervation.

In the vertebrate embryonic circulation, the subintestinal system: ventral aorta − heart − subintestinal vein (portal and mesenteric veins in adult) is the main channel of the venous system. In animals bearing gills, the heart develops and serves as a "portal" system for the branchial gut. It is long known that the hepatic portal veins of many animal species are pulsatile. The presence of cholinergic innervation was reported in several animals (Table 2). The ventral aorta of eel and trout were pulsatile and cholinergic innervation was shown (64). Whether or not the smooth muscle of the ventral aorta and the hepatic portal vein is developed as myoepithelium like the cardiac muscle is not known. Investigation from such a viewpoint is wanted.

The above stated findings and information well support our hypothesis. The parasympathetic cholinergic nerves originally distribute to the inner tube to regulate the movement of smooth muscle of the digestive tube. It is probable that in the cardiovascular system, they likewise distribute mainly to the vessels of the visceral part and the heart. We also assume that the muscle of these vessels and the heart are derived from common origin with muscle of the digestive tube. It is likely that these muscle cells share a property necessary for the establishment of innervation with cholinergic nerves.

7.3. The efferent pathway of the cholinergic innervation

The efferent pathway of the cholinergic innervation to the veins of the digestive tube has not been fully clarified. Nakazato et al. (16) demonstrated that in in vivo experiments of dogs, stimulation of the greater splanchnic nerves, but not of the vagus nerve, evoked an atropine-sensitive cholinergic response in tension recordings from the middle segment of IVC. Subsequently, a similar finding was observed in recordings from the portal vein (25). Thus the efferent pathway of the cholinergic innervation of these veins appears to be mostly, if not entirely, mediated through the splanchnic nerves. The efferent fibers contained in these nerves are generally regarded as "sympathetic", that is, of thoraco-lumbar outflow, preganglionic fibers. This may apparently contradict our hypothesis regarding the cholinergic innervation to these veins as a component of the "parasympathetic" nervous system. There is also some evidence indicating a cholinergic (atropine-sensitive) mechanism mediated through the splanchnic nerves to the digestive tract, distinct from the vagal cholinergic mechanism (1, 65, 66).

According to Pick (67), the most striking feature morphologically in the evolution of the autonomic nerves is the initial preponderance of the dorsal outflow, which gradually shifts in favor of the ventral outflow (Fig. 8). At first, all autonomic nerves emerge in a segmental fashion through all dorsal roots as seen in the amphioxus (Fig. 8a). Subsequently, in the cyclostomata (Fig. 8b), the dorsal outflow is restricted to the head as the vagus nerve complex. The gut is mainly innervated by the vagus, which extends far back along the intestine. There is little contribution of fibers from the spinal nerves to the alimentary canal, since as Young (68) suggested, this has

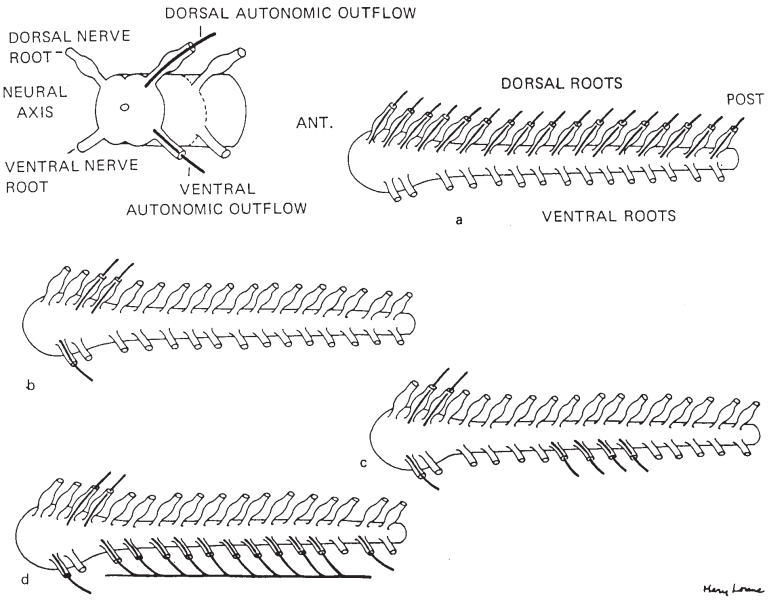

Fig. 8. Scheme showing 4 stages of the phylogenetic development of the outflow of autonomic nerves. Stage a: all autonomic nerves emerge through dorsal spinal roots. Stage b: autonomic outflow is restricted to the anterior end as vagus nerve complex, added by a ventral outflow, the pupillary motor nerve. Stage c: anterior outflow remains as in stage b and is augmented by a segmental ventral outflow in the trunk. Stage d: dorsal outflow is unchanged; more segments are added to the ventral outflow which, except for the extreme anterior and posterior levels, become connected, forming the sympathetic trunk. (Reproduced from Ref. 67).

no mesentery, being attached only at its cranial and caudal ends. While the cranial vagal outflow remains, autonomic nerves grow out in a segmental fashion through the ventral roots. Thus, in some sharks (Fig. 8c), this ventral outflow is confined to the head as the pupillomotor nerve and to a variable number of segments of the trunk. In bony fish (Fig. 8d), the branches of the ventral autonomic outflow connect with each other to form the sympathetic trunk.

According to Burnstock (1), in lower vertebrates (elasmobranches and teleost fish), the vagal influence doest not extend beyond the stomach or the anterior intestine, and the sympathetic (thoraco-lumbar) supply to the intestine is a mixture of predominantly excitatory (probably cholinergic) and some inhibitory (probably adrenergic) fibers. In higher vertebrates the vagal influence extends down to the gut, and the sympathetic nerves become predominantly inhibitory. One interesting possibility is then that the excitatory cholinergic innervation to the veins of the visceral part, observed in the dog, is a remnant of the "spinal dorsal outflow" to the gut.

8. Remarks

In this article, the importance of morphogenetical background in understanding the cholinergic innervation in the cardiovascular system has been stressed. According to stages in the evolution of animals, both the anatomical paths for a nerve to reach its targets and the distribution of the target tissues may vary. This issue may well be demonstrated in the existence of cholinergic innervation in the dog IVC, the lack of the innervation in the splenic vein in the portal venous system, and the findings by Hirakow and Miki (50) in the comparative study on the cardiac nerves. The last study also indicates the phylogeny of the autonomic nervous system. A systematic survey of drug responses of blood vessels will be useful as a good indicator of such regional differences of blood vessels reflecting their phylogeny.

The importance of selecting an appropriate material or species for study should also be pointed out. According to Ogawa (51), the important finding is the following: The crossing of the cardiac nerves going to the ventricles was clearly observed only in the beaked whale *Berardius bairdii Stejneger*, which is a primitive species among whales, but not so definitely in two other sorts of whales examined. Hirakow and Miki (50) also emphasized the importance of selecting species to be examined. In our study on the IVC, we might not have been able to detect the cholinergic innervation, if the rabbit, rather than dog, was selected.

Acknowledgments

The authors are deeply grateful to the late Prof. Ryoji Ura, Tohoku University School of Medicine; the late Prof. Shigeo Miki, the Tokyo National University of Fine Arts and Music; and the late Prof. Reiji Hirakow, Saitama Medical School for their kind help over many years. We also wish to thank our colleagues of the Department of Pharmacology, Nagoya University School of Medicine, where studies on the dog venous system were performed.

References

1 Burnstock G. Evolution of the autonomic innervation of visceral and cardiovascular systems in vertebrates. Pharmacol Rev. 1969; 21:248–324.
2 Bell CH, Burnstock G. Cholinergic vasomotor neuroeffector junctions. In: Bevan JA, Furchgott RF, Maxwell RA, Somlyo AP, editors. Physiology and pharmacology of vascular neuroeffector systems. Basel: Karger; 1971. p. 37–46.
3 Bevan JA, Brayden JF. Nonadrenergic neural vasodilator mechanisms. Circ Res. 1987;60:309–326.
4 Kalsner S. Cholinergic constriction in the general circulation and its role in coronary artery spasm. Circ Res. 1989;65:237–257.
5 Bell C. Cholinergic vasodilator mechanisms. In: Bennett T, Gardiner SM, editors. The autonomic nervous system. Vol. 8. Nervous control of blood vessels. Amsterdam: Harwood Academic Publishers; 1996. p. 59–74.
6 Broten TP, Miyashiro JK, Moncada S, Feigl EO. Role of endothelium-derived relaxing factor in parasympathetic coronary vasodilation. Am J Physiol. 1992;262:H1579–H1584.
7 McMahon TJ, Hood JS, Kadowitz PJ. Pulmonary vasodilator response to vagal stimulation is blocked by Nω-nitro-L-arginine methyl ester in the cat. Circ Res. 1992;70:364–369.
8 Matsukawa K, Shindo T, Shirai M, Ninomiya I. Nitric oxide mediates cat hindlimb cholinergic vasodilation induced by stimulation of posterior hypothalamus. Jpn J Physiol. 1993;43:473–483.
9 Loke KE, Sobey CG, Dusting GJ, Woodman OL. Requirement for endothelium-derived nitric oxide in vasodilatation produced by stimulation of cholinergic nerves in rat hindquarters. Br J Pharmacol. 1994;112:630–634.
10 Toda N, Okamura T. Mechanism underling the response to vasodilator nerve stimulation in isolated dog and monkey cerebral arteries. Am J Physiol. 1990;259:H1511–H1517.
11 Toda N, Okamura T. The pharmacology of nitric oxide in the peripheral nervous system of blood vessels. Pharmacol Rev. 2003;55:271–324.
12 Shigei T, Ishikawa N, Ichikawa T, Tsuru H. Differences in the response of three embryologically distinct segments of the isolated canine posterior vena cava to vasoactive substances. Blood Vessels. 1978;15:157–169.
13 Shigei T, Ichikawa T, Ishikawa N, Uematsu T, Tsuru H. Functional venous characteristics in relation to embryology. In: Bevan JA, Fujiwara M, Maxwell RA, Mohri K, Shibata S, Toda N, editors. Vascular Neuroeffector Mechanisms: 4th International Symposium. New York: Raven Press; 1983. p. 203–208.
14 Shigei T, Ishikawa N, Hirakow R. An embryological aspect of cholinergic innervation in the cardiovascular system (abstract). Jpn J Pharmacol. 1992;58 Suppl II:302P.
15 Ichikawa T, Ishikawa N, Shigei T. Innervation of the canine inferior vena cava. Distribution of adrenergic and cholinergic excitatory fibers among the embryologically distinct segments. Blood Vessels. 1979;16:155–162.
16 Nakazato Y, Ohga A, Shigei T, Uematsu T. Extrinsic innervation of the canine abdominal vena cava and the origin of cholinergic vasoconstrictor nerves. J Physiol. 1982;328:191–203.
17 Furuta T, Hayakawa A, Iida N, Inagaki A, Shigei T. Distribution of cholinesterase in canine venous system. Jpn J Pharmacol. 1987;43:237–241.
18 Yoshioka K. Hepatic veins belong to “the veins of the digestive tube” in the dog. Jpn J Pharmacol. 1988;46:424–427.
19 Yoshioka K, Furuta T, Hayakawa A, Ishikawa N, Shigei T. Excitatory cholinergic innervation in canine portal and mesenteric veins. Am J Physiol. 1988;255:H288–H294.
20 Shigei T. An embryological view about cholinergic innervation in the cardiovascular system. In: Sperelakis N, Wood JD, editors. Frontiers in smooth muscle research. New York: Wiley-Liss; 1990. p. 591–593.
21 Shigei T, Tsuru H, Ishikawa N, Yoshioka K. Absence of endothelium in invertebrate blood vessels: Significance of endothelium and sympathetic nerve/medial smooth muscle in the vertebrate vascular system. Jpn J Pharmacol. 2001;87:253–260.
22 Miki S. Seimei no keitaigaku. <6> Junkan-kei. Sougou kango, 1979;14:91–139. (in Japanese) [(reproduced in Miki S. Seimeikeitai no shizenshi, Vol. 1 Kaibougaku-ronshu, Tokyo: Ubusuna Shoin; 1989. p. 181–229. (in Japanese)]
23 Miki S. Genesis of the splenic vein. In: Bevan JA, Fujiwara M, Maxwell RA, Mohri K, Shibata S, Toda N, editors. Vascular Neuroeffector Mechanisms. 4th International Symposium. New York: Raven Press; 1983. p. 195–201.
24 Langman J. Medical embryology. 3rd ed. / 3rd Asian ed. Baltimore: Williams & Wilkins / Tokyo: Igaku Shoin; 1975.
25 Uematsu T, Takiguchi Y, Nakashima M. Extrinsic innervation of the canine superior vena cava, pulmonary, portal and renal veins. Angiology. 1984;35:486–493.
26 Furuta T, Shigei T. A regional difference in endothelium-derived relaxation responses to acetylcholine in the canine venous system. Jpn J Pharmacol. 1987;44:207–210.
27 Milnor WR, Sastre A. Cholinergic receptors and contraction of smooth muscle in canine portal vein. J Pharmacol Exp Ther. 1988;245:244–249.
28 Feletou M, Hoeffner U, Vanhoutte PM. Endothelium-dependent relaxing factors do not affect the smooth muscle of portal-mesenteric veins. Blood Vessels. 1989;26:21–32.
29 Furuta T, Ishikawa N, Shigei T. Regional variation in β-adrenoceptor-mediated relaxation of canine veins. Blood Vessels. 1986;23:236–245.
30 Tsuru H, Ishikawa N, Shigei T. Responsiveness of isolated dog veins to bradykinin and other bioactive peptides: distribution of sensitivity to bradykinin and possible correlation with genesis of the venous system. Blood Vessels. 1976;13:238–248.
31 Ishikawa N, Ichikawa T, Shigei T. Possible embryogenetical differences of the dog venous system in sensitivity to vasoactive substances. Jpn J Pharmacol. 1980;30:807–818.
32 Hamilton WJ, Boyd JD, Mossman HW. Human embryology. 4th ed. Baltimore: Williams & Wilkins; 1972. p. 560.
33 Romer AS, Parsons TS. The vertebrate body, shorter version. 5th ed. Philadelphia, London, Toronto: W.B. Saunders; 1978: p. 31–32, p. 203, p. 218–219, p. 326.
34 Ishikawa N. [Effects of vasoactive substances on isolated canine posterior vena cava.] Nagoya Igaku. 1976;99:11–17 (text in Japanese with English abstract).
35 Takaoka K. [Development of blood vessels of pro- and opisthonephros of *Hynobius naevius* (a salamander).] Acta Anat Nippon. 1956;31:349–376 (text in Japanese with Esperanto abstract).
36 Bennett T, Malmfors T. The vasomotor innervation of the inferior vena cava of the domestic fowl (*Gallus gallus domesticus* L.) - II. Functional observations. Gen Pharmacol. 1975;6:49–51.
37 Takeshige Y. Existence of spindle venules in portal system of mesentery and their functional significance. In: Proceedings of

the 8th International and 19th Brazilian Congress of Angiology (Progress on Angiology). 1976. p. 465–472.
38 Yoshioka K, Hayakawa A, Furuta T, Ishikawa N, Shigei T. Distinctive characteristics of the splenic vein in the dog. Its morphological and pharmacological discontinuities with the portal vein and splenic capsule. Blood Vessels. 1988;25:273–284.
39 Miki S. [The genetic relation of the spleen to the secondary gastro-intestinal vein in chick embryo.] Acta Anat Nipp. 1965;40: 329–341. (text in Japanese with English abstract)
40 Roy CS. The physiology and pathology of the spleen. J Physiol. 1881;3:203–228.
41 Barcroft J, Nishimaru Y. Cause of rhythmical contraction of the spleen. J Physiol. 1932;74:299–310.
42 Nilsson S, Grove DJ. Adrenergic and cholinergic innervation of the spleen of the cod, *Gadus morhua*. Eur J Pharmacol. 1974;28: 135–143.
43 Pettersson K, Nilsson S. Nervous control of the branchial vascular resistance of the Atlantic cod, *Gadus morhua*. J Comp Physiol. 1979;129:179–183.
44 Berger PJ. The vagal and sympathetic innervation of the isolated pulmonary artery of a lizard and a tortoise. Comp Gen Pharmacol. 1972;3:113–124.
45 Bolton TB. Studies on the longitudinal muscle of the anterior mesenteric artery of the domestic fowl. J Physiol. 1968;196:273–281.
46 Kalsner S. Cholinergic mechanisms in human coronary artery preparations: Implications of species differences. J Physiol. 1985;358:509–526.
47 Miki S. [Phylogeny of the vegetative nervous system – Origin of so-called parasympathetic and sympathetic nerves.] Seitai no kagaku. 1988;39:618–623. (in Japanese) [reproduced in Miki S. Seimeikeitai no shizenshi, Vol. 1 Kaibougaku-ronshu. Tokyo: Ubusuna Shoin; 1989. p. 354–359. (in Japanese)]
48 Otorii T. Pharmacology of the heart of *Entosphenus japonicus*. Acta Medica Biologica. 1953;1:51–59.
49 Morris JL, Nilsson S. The circulatory system. In: Nilsson S, Holmgren S, editors. Comparative physiology and evolution of the autonomic nervous system. Chur, Switzerland: Harwood Academic Publishers; 1988. p. 193–246.
50 Hirakow R, Miki S. Phylogenetic observations on the cardiac nerves of vertebrates. Proc Japan Acad. 1971;47:225–230.
51 Ogawa T. On the cardiac nerves of some Cetacea, with special reference to those of *Berardius bairdii Stejneger*. Sci Repts Whales Res Inst. 1952;7:1–22.
52 Licata RH. The human embryonic heart in the ninth week. Am J Anat. 1954;94:72–125.
53 Hirakow R. [Basic pattern of the cardiac nerves in vertebrates.] Brain Nerve. 1987;39:417–423. (in Japanese)
54 Wiggers CJ. Physiology in health and disease, 8th Edition, Philadelphia: Lea and Febiger; 1949. p. 643.
55 Irisawa H, Caldewell WH, Wilson MF. Neural regulation of atrioventricular conduction. Jpn J Physiol. 1971;21:15–25.
56 Shepherd JT, Vanhoutte PM. The human cardiovascular system. Facts and concepts. New York: Raven Press; 1979. p. 123.
57 Ura R. Lecture note on anatomy at Iwate Medical University. 1972.
58 Frist S, Stemerman B. Arterial growth and development. In: Bevan JA, Burnstock G, Johansson B, Maxwell RA, Nedergaard OA, editors. 2nd International Symposium on Vascular Neuroeffector Mechanisms. Basel: Karger; 1976. p. 19–27.
59 Le Lièvre CS, Le Douarin NM. Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. J Embryol Exp Morph. 1975;34:125–154.
60 Blomhoff R, Wake K. Perisinusoidal stellate cells of the liver: important roles in retinol metabolism and fibrosis. FASEB J. 1991;5:271–277.
61 Hirakow R. The vertebrate heart in phylogenetic relation to the prochordates. In: Duncker HR, Fleischer G, editors. Fortschritte der Zoologie, Band 30. Vertebrate Morphology. Stuttgart, New York: Gustav Fischer Verlag; 1985. p. 367–369.
62 Nakao T. An electron microscopic study of the circulatory system in *Nereis japonica*. J Morph. 1974;144:217–235.
63 Ljung B. Nervous and myogenic mechanisms in the control of a vascular neuroeffector system. Acta Physiol Scand Suppl. 1970;349:33–68.
64 Kilby S, Burnstock G. Comparative pharmacological studies of isolated spiral strips of large arteries from lower vertebrates. Comp Biochem Physiol. 1969;28:307–319.
65 Furnet J, Snape WJ Jr, Cohen S. Sympathetic control of lower esophageal sphincter function in the cat. J Clin Invest. 1979;63: 562–570.
66 Gonella J, Niel JP, Roman C. Sympathetic control of lower esophageal sphincter motility in the cat. J Physiol. 1979;287:177–190.
67 Pick J. The autonomic nervous system. Morphological, comparative, clinical and surgical aspects. Chapter 21, phylogenetic considerations. Philadelphia, Tronto: Lippincott; 1970.
68 Young JZ. The life of vertebrates, 2nd ed. Oxford: Oxford University Press; 1962. p. 173.
69 Campbell G. Autonomic innervation of the pulmonary vascular bed in a toad (*Bufo marinus*). Comp Gen Pharmacol. 1971;2:287–294.
70 Holman ME, McLean A. The innervation of sheep mesenteric veins. J Physiol. 1967;190:55–69.
71 McCalden TA, Roddie IC. Excitatory cholinergic fibres to longitudinal smooth muscle in bovine mesenteric veins (Proc.). Ir J Med Sci. 1971;140:48.
72 Tsuru H, Mutsukado Y, Shigei T, Sasa M. Evidence for the excitatory cholinergic innervation in the rabbit portal vein. Jpn J Pharmacol. 1994;66:393–396.
73 Kalsner S, Quillan M. Cholinergic contraction to field stimulation in coronary arteries of cattle. J Pharmacol Exp Ther. 1989; 249:785–789.
74 Botár J. Phylogenetic evolution of the vegetative nervous system. Acta Neurovegetativa. 1967;30:342–349.