A Regional Difference in Endothelium-Dependent Relaxation Responses to Acetylcholine in the Canine Venous System†

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Abstract—Endothelium-dependent relaxations of canine veins isolated from 15 different sites were examined. Acetylcholine (ACh, 10^{-10}–10^{-6} M) caused marked endothelium-dependent relaxations in the external jugular vein, superior vena cava, brachiocephalic vein, segment A (supradiaphragmatic portion) and D (infrarenal portion) of the inferior vena cava. However, only contractile responses were induced by ACh in the portal, mesenteric veins and the segment C of the inferior vena cava (between liver and renal veins) with or without endothelium. The other 7 veins showed only small endothelium-dependent relaxations (10–20%). These results indicated that the endothelium-dependent responses of canine veins to ACh are regionally different.

In recent years, much attention has been paid to the role of the endothelium in relaxation responses of vascular smooth muscles (1, 2). A variety of substances, including acetylcholine (ACh), bradykinin, A23187, thrombin and substance P, are known to cause a similar type of vasorelaxation via the endothelium (1, 2). Evidences of the endothelium-dependent relaxation are documented in arterial tissues of many kinds of animals (1, 2). However, there are only a few reports describing the endothelium-dependent responses of veins (3, 4). In previous papers, we repeatedly indicated that the canine venous system consists of veins characteristically different in pharmacological responses (5–9). Therefore, in the present study, distribution of endothelium-dependent relaxation responses in the venous system was investigated by using 15 canine veins selected systematically (5, 6).

Adult mongrel dogs of either sex (body weight, 7–15 kg) were anesthetized with sodium pentobarbital (35 mg/kg). After heparin was administered to prevent the blood clotting over the inner surface of vessels, segments of veins were carefully removed from 15 different sites (5, 6). The veins examined were the external jugular, cephalic, brachiocephalic, azygos, pulmonary, portal, mesenteric, splenic, renal, femoral and lateral saphenous veins, the superior vena cava. The inferior vena cava was divided into 4 segments (7): the supradiaphragmatic portion (segment A), the portion between liver and the renal veins (segment C), and the infrarenal portion (segment D) were examined. Segment B, the intrahepatic portion, has similar pharmacological characteristics to segment C (7) and was not used in the present study. Isolated veins were placed immediately into Krebs' bicarbonate solution of the following composition (mM): NaCl, 119; KCl, 3.7; $CaCl_2$, 2.5; KH_2PO_4, 1.18; $MgSO_4$, 1.17; $NaHCO_3$, 24.9; glucose, 11.1, pH 7.4. Veins were cleaned of connective tissue and cut into helical strips (2–4 mm in width, 8–9 mm in length). Longitudinal strips (1–2 mm in width, 5–8 mm in length) were also prepared from the portal, mesenteric vein and segment C of the inferior vena cava. These procedures were done carefully not to injure the endothelial layer. In order to remove the endothelium, the inner surface of the veins was gently rubbed by a

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cotton wig. During an experiment, the removal of the endothelium was determined by disappearance of the relaxation responses to A23187 (10^{-7} M). Presence or removal of the endothelium was also confirmed by silver staining and microscopic observation after mechanical study. The preparations were suspended vertically in a 10 ml organ bath filled with Krebs' bicarbonate solution maintained at 37°C and bubbled with a gas mixture of 95% O_2 and 5% CO_2. Optimal resting tensions, which produced the maximum contractions to methoxamine, ranged from 0.5 g to 1.5 g. Isometric tension was measured by force-displacement transducer (Kyowa Dengyo, 120T–10B) connected to an amplifier (Kyowa Dengyo, DPM1N) and traced on a paper recorder. Preparations were allowed to equilibrate for at least 60 min before the experiments were started.

Drugs used in this study were methoxamine hydrochloride (Nippn Shinyaku), acetylcholine hydrochloride (Daiichi Seiyaku), A23187 (Sigma) and bovine thrombin (Mochida Seiyaku).

Relaxation responses of veins with or without endothelium to ACh (10^{-10}–10^{-6} M) were examined after preconstriction with methoxamine (5×10^{-6}–10^{-5} M). The preconstriction level of 15 veins ranged from 50% (azygos vein) to 70% (longitudinal strip of the mesenteric vein) of the maximal contractions induced by methoxamine. ACh was added cumulatively, and the maximal relaxations, which were expressed by the percent values of induced active tone, were obtained. ACh caused marked relaxation in an endothelium intact external jugular vein but not in the endothelium denuded one (Fig. 1A). Brachiocephalic vein, superior vana and segment A and D of the inferior vena cava also showed relatively large endothelium-dependent relaxations (38–48%), whereas the cephalic, pulmonary, azygos, renal, femoral and saphenous veins showed only small endothelium-dependent relaxations (10-20%) (Table 1). The splenic vein showed an endothelium-dependent relaxation response to ACh, as well as an endothelium-independent one (3). Only contractile responses to ACh were seen in both transverse and longitudinal strips of the portal, mesenteric veins and

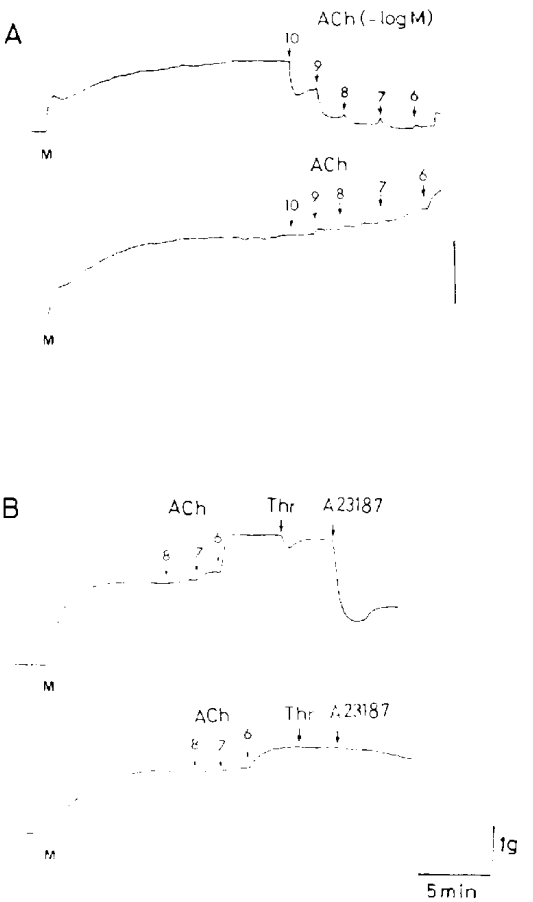

Fig. 1. Endothelium-dependent responses of canine veins. A: external jugular vein, B: transverse strip of portal vein. The upper and lower traces of each panel are tension recordings of endothelium (+) and endothelium (−) preparations, respectively. M: methoxamine (10^{-5} M). Arrows show the applications of ACh (−log M), thrombin (Thr, 1 unit/ml) and A23187 (10^{-8} M).

segment C of the inferior vena cava regardless of the presence or absence of the endothelium. However, these veins showed obvious endothelium-dependent relaxation resposes to thrombin or A23187 (see Fig. 1B).

Previously, De Mey and Vanhoutte (3) described the different nature of the endothelium of some canine veins in pharmacological responses from those of arteries. They concluded that the endothelium of veins mainly mediates the contractile responses rather than relaxation ones, because ACh-

Table 1. Endothelium-dependent relaxations of canine veins induced by acetylcholine

Veins			Maximum relaxations (%)	Preconstriction (%)	(N)
Cephalic			10.0±1.8	60.3±6.6	(4)
External jugular			89.8±4.0	58.6±4.4	(6)
Brachiocephalic			40.0±7.2	52.0±1.5	(5)
SVC			44.3±8.0	55.8±2.5	(5)
Pulmonary			16.5±3.0	56.0±3.2	(5)
Azygos			11.8±3.5	50.3±0.9	(4)
IVC	A		37.5±3.9	53.0±1.5	(4)
	C	(H)	contraction	62.4±4.1	(5)
		(L)	contraction	60.3±2.7	(4)
	D		41.8±8.8	57.5±1.4	(5)
Portal		(H)	contraction	59.4±2.8	(5)
		(L)	contraction	64.0±3.2	(5)
Renal			20.0±7.3	52.0±1.9	(5)
Mesenteric		(H)	contraction	53.0±2.6	(4)
		(L)	contraction	70.4±2.1	(6)
Splenic			24.0±4.2#	58.5±3.5	(6)
Femoral			17.8±4.8	57.8±1.5	(5)
Saphenous			15.0±2.2	64.8±3.1	(5)

N: number of preparations, H: helical strips, L: longitudinal strips, SVC: superior vena cava, IVC: inferior vena cava, A: segment A, C: segment C, D: segment D. See text for details. Each value is a mean±S.E. Maximal relaxations were obtained by cumulative addition of acetylcholine following the contraction with methoxamine (5×10^{-6}–10^{-5} M). The preconstriction levels were percent values of the maximum contractions. #: Splenic veins showed endothelium-independent relaxation to ACh, as well as endothelium-dependent ones. These values are thus, the sum of both.

induced relaxations of saphenous or femoral veins through the endothelium was so small and because the endothelium mediated apparant contractile responses to arachidonic acid or thrombin (3). However, in the present study using 15 veins, there were certain veins showing a large endothelium-dependent relaxation response to ACh, for instance, the external jugular and brachiocephalic vein, superior vena cava and segment A and D of inferior vena cava, probably indicating the importance of the endothelium in the pharmacological responses of these veins.

The present study also showed a marked regional difference in the responses of veins to ACh. Some veins (portal, mesenteric veins and segment C of the inferior vena cava) showed only contractile responses to ACh, while other veins showed the endothelium-dependent relaxations of various degrees (Table 1). The preconstrictions evoked by methoxamine slighly varied among the veins, but the regional difference in endothelium-dependent relaxations were not related to the variation of the preconstriction levels (see, for instance, the results of jugular vein and azygos veins in Table 1). Acetylcholine is known to cause the contractile responses in the veins showing the endothelium-dependent relaxations in the present study (6, 9). However, the influence of such contractions on the observed relaxation responses seemed unlikely, because ACh-induced contractile responses of these veins occurred at higher concentration than 10^{-6} M (6, 9), whereas the endothelium-dependent relaxations evoked by ACh were seen at lower concentrations than this, and maximum responses were obtained around 3×10^{-7} M. Thus, the present study indicated that the endothelium-dependent relaxation responses to ACh are regionally different in the canine venous system. As to the portal, mesenteric veins and segment C of the inferior vena cava, there is, at present, no adequate explanation for the absence of the endothelium-dependent relaxation to ACh in spite of a marked one to thrombin or A23187. However, it is of interest that the three veins showing the

contractile responses are different from the other veins either in their embryological origin (5–8) or by the presence of cholinergic excitatory innervation (8, 9).

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