Distribution of Cholinesterase in Canine Venous System

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Abstract—Cholinesterase (ChE) activity was examined in 15 veins from dogs by pharmacological and histochemical procedures. Potentiation of acetylcholine (ACh)-induced contractile response by neostigmine was observed in helical and longitudinal strips of portal, mesenteric veins and middle segment of the inferior vena cava, but not in the other 12 veins. Histochemical studies with an electron microscope (the method of Karnovsky-Roots) revealed that ChE activity was identified as high density granules around the smooth muscle cells only in the former three veins. The visible ChE activity was abolished by pretreatment with neostigmine.

In previous papers, we extended the view that the canine veins can be classified into two groups: veins of the body wall and those of the digestive tube (1–4). The latter group includes portal and mesenteric veins and the middle portion of the inferior vena cava (segment B–C, see below). These three veins are embryologically related to the digestive tube, being characterized by the presence of massive longitudinal muscles, spontaneous rhythmic contractile activity, as well as cholinergic excitatory innervation (1–4). Some evidences of the innervation obtained by in vitro and in vivo experiments were reported (3, 5–7). Additional findings were reported by Uematsu et al. (8).

The present study was undertaken to investigate the distribution of cholinesterase (ChE) in the whole canine venous system in order to determine whether or not cholinergic nerves are localized only in the veins of the digestive tube. Isolated venous strips were examined for a potentiating effect of neostigmine on acetylcholine (ACh)-induced contraction and for a histochemical detection of cholinesterase activity in electronmicroscopy.

Adult mongrel dogs of either sex (8–15 kg) were anesthetized with sodium pentobarbital (35 mg/kg, i.v.). Segments of veins were removed from 15 different sites of the venous system and then immediately placed in Krebs’ bicarbonate solution. The composition of the solution was as follows (mM): NaCl, 119; KCl, 4.7; CaCl2, 2.5; KH2PO4 1.2; MgSO4, 1.17; NaHCO3, 24.9; glucose, 11.1. The pH of the solution was 7.4. Veins examined were external jugular, cephalic, brachiocephalic, azygos, pulmonary, portal, mesenteric, splenic, renal, femoral, lateral saphenous veins, superior vena cava and inferior vena cava. The inferior vena cava was divided into 4 segments (3–4). Supradiaphragmatic portion (segment A), the portion between liver and renal veins (segment C), and infrarenal portion (segment D) were studied. Segment B, the intrahepatic portion, has the same properties as segment C, except that its spontaneous rhythmic activity is more marked than that of segment C.

Potentiation of ACh-induced contraction by neostigmine: Isolated veins were cleaned of connective tissues and cut into helical strips (H), 2–4 mm in width, 8–9 mm in length. Longitudinal strips (L), 1–2 mm in width and 5 mm in length, were also prepared from the portal and mesenteric veins and segment C of the inferior vena cava, which are rich in longitudinal muscles. Regarding the jugular vein, a separate study indicated that this vein showed a marked endothelium-dependent inhibition of the con-
tractile response to ACh (T. Furuta et al., unpublished observation). Therefore, experiments with jugular vein were carried out after removal of the endothelium. 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% O₂ and 5% CO₂. Resting tensions ranged from 0.5 g to 1.5 g according to the width of the strips. Isometric tensions were measured by a force-displacement transducer (Kyowa Dengyo, 120T-10B). The preparations were allowed to equilibrate for 60 min before the experiments were started.

ACh was applied cumulatively, and a concentration-response curve was obtained. This procedure was then repeated in the presence of 10⁻⁷ M and 3×10⁻⁷ M of neostigmine. Neostigmine caused a parallel leftward shift of the curve in helical and longitudinal strips of the portal and mesen-

![Graphs showing effects of neostigmine on ACh-induced contractile response of canine veins.](image)

**Fig. 1.** Effects of neostigmine on ACh-induced contractile response of canine veins. A: longitudinal strips of segment C of inferior vena cava, B: helical strips of mesenteric vein, C: external jugular vein, D: renal vein, E: femoral vein. ●: control responses, △: with 10⁻⁷ M neostigmine, □: with 3×10⁻⁷ M neostigmine. Contractions are expressed by % values of the maximum contractions in control responses (segment C: 6.1±1.2 g/mm width, mesenteric vein: 1.0±0.29 g/mm width, external jugular vein: 0.19±0.07 g/mm width, renal vein: 0.46±0.14 g/mm width, femoral vein: 1.1±0.24 g/mm width). Each value is a mean±S.E. (n=6–8).
Fig 2. Histochemically detected cholinesterase activity in canine veins (arrows). Longitudinal muscle layers of portal vein (A) and segment C of inferior vena cava (B). C. X-ray spectrum of a dense granule obtained in portal vein counted for 300 sec. (Vertical full scale=256 counts). Note the high peaks of Fe and Cu. Au peak is caused by the grid.
teric veins and segment C of the inferior vena cava. No such shift of the curve was observed in the other 12 veins (Fig. 1).

**Histochemical study:** The identification of ChE activity was performed by the method of Karnovsky-Roots (9). The isolated tissues were washed with 0.44 M sucrose solution, cut into small pieces (2x3 mm), and fixed with 4% formaldehyde, adjusted to pH 6.8, at low temperature (2–4°C) for 3 hr. After rinsing with 0.44 M sucrose solution, the tissues were immersed into the medium of Karnovsky-Roots containing acetylthiocholine iodide (0.5 mg/ml), sodium citrate (5 mM), CuSO₄ (3 mM), and potassium ferricyanide (0.5 mM), for 45 min at 20°C. The ChE activity was expected to appear as a visible copper ferrocyanide precipitate (9). In some experiments, 10⁻⁴ M neostigmine, was present in the incubation medium.

The stained tissues were further fixed with 2.5% glutaraldehyde buffered with 0.2 M Millonig’s phosphate buffer (pH 7.4) for 2 hr, dehydrated in graded ethanols, and embedded in Epon 812. Epoxy blocks were sectioned with an LKB ultratome into 90–150 m/c thick sections. Some of these sections were stained by uranyl acetic acid and observed with a Nihon Denshi 1000X electronmicroscope. Other sections were mounted on Maxtaform gold (Au) grids, HR 24 (Glu- ticularis, London), for the electron probe X-ray analysis without electron staining (10). These were observed with a Hitachi H-800 electronmicroscope equipped with Kevex 7000-Q energy dispersion X-ray microanalyzer. The accelerating voltage was 100 kv and the beam current was 10⁻¹⁰ ampere.

Fifteen sites of veins were examined. Figure 2 shows typical electron microscopic findings in longitudinal muscle layers of the portal vein and segment C of the inferior vena cava. The ChE activity was detected as high density granules located around the smooth muscle cells. Neostigmine treatment completely abolished the activity. As shown in Fig. 2C, electron probe X-ray analysis revealed that the main peaks detected in these granules were Cu and Fe. (Au peak is caused by the grid.) Similar pattern of Cu and Fe containing granules were also observed in the mesenteric vein. However, in the other 12 veins, such visualized ChE activity could not be detected.

The results of the present pharmacological and histochemical studies indicate that detectable cholinesterase activity is localized in the three veins of the digestive tube: portal and mesenteric veins and segment C (and certainly segment B) of the inferior vena cava. These findings add further support to our view that the smooth muscles of these veins are embryogenetically related to the intestinal smooth muscles, so they receive a cholinergic excitatory innervation in common with the latter (2–4). However, demonstration of cholinergic nerve fibers and analysis of substrate specificity of the enzyme await further studies.

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