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Cardiotopic Organization of the Functionally Associated Axons Within the Cervical Vagus Nerves That Project to the Ventricles of the Cat Heart

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Introduction: Data obtained in the cat model indicate that there is a regional organization of functionally associated vagal preganglionic cardioinhibitory neurons in the CNS, and vagal postganglionic cardioinhibitory neurons within the heart. These data have been confirmed, in part, in other models, including the rat, dog, pig, primates, and humans. Succinctly stated, these data indicate that anatomically separated and functionally selective cardioinhibitory neurons are found in both the CNS and the intrinsic cardiac nervous system. These neurons are interconnected via the vagus nerves. In the heart parasympathetic postganglionic cardioinhibitory neurons are found within fat pads on the epicardial surface as well as within the myocardium. A total of three separate intracardiac ganglia innervate the left ventricle; however, the interventriculoseptal (IVS) ganglion provides the major source of innervation to the anterior surfaces of both the left and right ventricles. Although considerable evidence has been accumulated that describes the origin, distribution, and functions of cardioinhibitory neurons within the brain and within the heart, much less morphologic information is available on the main neural circuit that interconnects these two sites; ie, the vagus nerves. Electrical stimulation of the vagi has important effects upon cardiac function. The vagus nerves contain substantial populations of both myelinated and unmyelinated axons. By varying the parameters and methods of vagal stimulation, it is possible to *selectively* activate *either* myelinated or unmyelinated axons. Stimulation of either myelinated or unmyelinated vagal axons caused negative chronotropic, dromotropic, or inotropic cardiac effects; however, there are both *qualitative* and *quantitative* differences in the evoked cardiac effects depending upon which type of fiber was activated. If vagal axons projecting to different intracardiac targets are differentially *spatially organized* within the vagi, and/or differ in the *numbers* of myelinated or unmyelinated axons that participate in eliciting desired physiologic effects, then it should be possible to induce extraordinarily selective effects upon cardiac performance by selecting the appropriate stimulation parameters and/or geometry. The present experiments were designed to test the hypotheses that: (1) vagal axons projecting to the IVS ganglion are differentially *spatially organized* within the vagi; and/or (2) different *numbers* of myelinated or unmyelinated vagal axons project to the IVS ganglion, where they may participate in eliciting selective physiologic effects on the ventricles.

Methods: Cholera toxin beta subunit conjugated to horseradish peroxidase (CTB-HRP) was microinjected into the IVS or pericardial space of eight cats to retrogradely label axons in the cervical vagus nerves. Animals were anesthetized and perfused intravascularly with fixatives after a 4-day survival. Retrogradely labeled axons in the vagi were identified by a histochemical method. Tissues were subsequently processed for electron microscopic visualization of both right and left vagus nerves at 2,500× magnification. Overlapping digitized photomontages of approximately 2,500 images each were assembled for each nerve. Custom software was developed to localize and count retrogradely labeled myelinated or unmyelinated axons in each of four quadrants superimposed sequentially at 90-degree intervals. Raw data were normalized by conversion to percent of total labeling. Subsequent data were analyzed using ANOVA with significance at $P < .05$. If the F test was significant, least significant difference post hoc tests were performed.

Results: The cervical vagus nerves in the cat contain one large fascicle (A), one moderate-size fascicle (B) below fascicle A, and 0 to 4 significantly smaller fascicles, irregularly distributed. Quantitative data were restricted to fascicles A and B in order to have a large enough sample size to achieve statistical significance. The regional distribution of myelinated labeled axons within the four quadrants of fascicle A ($P < .004$), or unmyelinated labeled axons within fascicle B ($P < .05$), of the *left* vagus nerve was not random. In contrast, the regional distribution of either myelinated or unmyelinated labeled axons in fascicles A or B of the *right* vagus nerve is random ($P > .05$). There were no statistically significant differences between the number of myelinated versus unmyelinated labeled axons in either bundle A or B of both vagus nerves.

Conclusions: These data are consistent with the hypothesis that vagal axons projecting to some intracardiac targets are cardiotopically organized within the vagus nerve. Although the total numbers of labeled myelinated and unmyelinated axons observed in the vagi were not statistically significantly different, this remains a potential mechanism for vagal projections to other intracardiac targets. Therefore, precise electrical stimulation of selected quadrants of the vagi may potentially elicit potent effects on the ventricles without directly influencing cardiac rate or atrioventricular conduction.

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