Parasympathetic control of the heart. I. An interventriculo-septal ganglion is the major source of the vagal intracardiac innervation of the ventricles

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Although there has been substantial interest in the neuroanatomic innervation of the heart for a considerable period of time (25), the locations, projections, and functions of the intracardiac ganglia are incompletely understood. The number of ganglia that have been characterized in the heart varies across species and ranges from as low as 4 in the rat (9, 27) to as many as 10 in humans (5, 32). Although many intracardiac ganglia are found in the atria, ganglia can also be found on the ventricular surface of the heart in diverse species, including humans (5, 28); dogs (4); cows, sheep, deer, and porpoises (14); rabbits and monkeys (25); and pigs (6). In the cat, it was suggested that intracardiac ganglia are found exclusively within the atria (10); however, in a recent study (20), it has been shown that at least one ventricular ganglion is found in the cat heart. Because the cat is an important animal model in cardiovascular research, we have reexamined the distribution and counted the number of neurons contained within intracardiac ganglia throughout the cat heart.

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field, dark-field, and Nomarski differential interference contrast optics. The locations of intracardiac ganglia were plotted onto drawings of transverse sections taken through eight representative levels of the heart (Fig. 1).

Experiments to determine the origin(s) of ganglia innervating the ventricles were performed on nine cats of either sex. Cats were sedated with a mixture of ketamine (22 mg/kg im) and acepromazine (0.22 mg/kg im) and given atropine (0.05 mg/kg im) to reduce respiratory secretions. Anesthesia was induced and maintained with isoflurane by inhalation. Subsequently, the animal was intubated with a cuffed endotracheal tube, and a thoracotomy was performed. Positive end-expiratory pressure artificial respiration was maintained thereafter. Body temperature was maintained at 37°C with warming pads. The pericardium was incised and carefully retracted. In each of eight animals, a total of 10 μl of the retrograde tracer diamidino yellow (1.0% in ethylene glycol containing 2% DMSO) was injected into four sites on the anterior surface of either the left or right ventricle using a Hamilton syringe (Fig. 2). In a control animal, 10 μl of diamidino yellow was injected into the pericardial space but not into the myocardium. The pericardium was then closed, and the muscles and skin were sutured in layers. Before the end of surgery, all animals received torbugesic (0.2 mg/kg im) for pain. Postoperatively, all cats received the analgesic drug ketoprofen (1 mg/kg sc) and the antibiotic procaine penicillin G (30,000 U/kg sc) daily. Animals were killed under deep barbiturate anesthesia (pentobarbital sodium, 55 mg/kg) by intravascular perfusion with fixatives after a 3-day survival time as described above. Every third 50-μm transverse frozen section of the heart taken from the aortic arch to within 3 mm of the apex of the ventricle was mounted on gelatinized slides, coverslipped with 50% aqueous glycerol, and examined using FITC fluorescence optics. Only neurons with an identifiable nucleus (>200 magnification) were counted. Cell counts were multiplied by three to compensate for examination of every third section. Retrogradely labeled cells were identified by the presence of a yellow nucleus and a clear cytoplasm. For each ganglion, we determined the total number of neurons and the number of retrogradely labeled neurons. Statistical analyses of the data were performed using a one-way ANOVA. Homogeneity of variance was evaluated using the Levene test, and multiple comparisons were evaluated using Tukey’s multiple-range test when group variances were equal or Dunnett’s T3 post hoc test when group variances were unequal.

RESULTS

Microscopic analysis of serial sections through the heart immunocytochemically stained with an antibody to the general parasympathetic nervous system.
neuronal marker PGP 9.5 revealed eight separate regions that consistently contained multiple closely associated clusters of neurons that we have referred to as ganglia (Figs. 1 and 3). Two ganglia were found on the right atrium [the posterior atrial (PA) and sinoatrial (SA) ganglia], one ganglion was found on the left atrium [the atrioventricular (AV) ganglion], one ganglion was found within the interatrial septum [the interatrio-septal (IAS) ganglion], one ganglion was found on the right ventricle [the right ventricular (RV) ganglion], two ganglia were found on the left ventricle (the CV ganglion and the LV2 ganglion), and one ganglion was found within the interventricular septum [the interventriculo-septal (IVS) ganglion]. Most of the ganglia were found within fatty and connective tissues between the surface of the heart and cardiac muscle, or surrounding the great vessels, and were in close proximity to nerve bundles. In some cases, however, particularly within the IVS ganglion, substantial numbers of neurons were found embedded in areas of myocardium that were completely surrounded by cardiac muscle cells and had little or no surrounding fatty matrix.

The PA ganglion is found in a fat pad located between the superior vena cava and the aorta on the posterior medial aspect of the right atrium. It is one of the largest ganglia on the heart. The PA ganglion is also the most rostrally located intrinsic cardiac ganglion. It begins just superior to the common pulmonary artery and ends at the superior aspect of the left atrium. In cross section, the PA ganglion is found ventral to the pulmonary artery and dorsal to the aorta and right atrium. The PA ganglion is comprised of large teardrop-shaped or small linear clusters of neurons widely separated and completely surrounded by fatty connective tissue. Clusters may be composed of as many as 60 neurons.

The SA and AV ganglia are located within fat pads on the surfaces of the atria. The SA ganglion is found on the right atrium located at the junction of the right pulmonary veins and the superior vena cava. The cells in this ganglion lie adjacent to the atrial myocardium and are oriented in a columnar or teardrop-shape fashion along the external border of the atrial myocardium. Occasionally, small ganglia can be found within the atrial myocardium. The outer border of the ganglion is

![Fig. 2. Schematic illustrations of the locations (*) of retrograde tracer injections into the right ventricle (A) or left ventricle (B) of the cat heart. Abbreviations as in legend to Fig. 1.](http://jap.physiology.org/DownloadedFrom)}
The AV ganglion is located at the junction of the inferior vena cava and the inferior left atrium. The cells in this ganglion are found in several oval clusters dispersed in connective and fatty tissue (21).

The CV ganglion is found in a fat pad located at the cranioventricular margin of the left ventricle. The rostral aspect of the ganglion originates at the ventral left lateral aspect of the conus trunci. Caudally, this ganglion begins to migrate dorsally toward the dorsal left lateral aspect of the right ventricle. The CV ganglion is found in a large mass of fatty tissue containing scattered clusters of ganglia comprised of a few cells (18, 20).

The RV ganglion is a relatively small ventricular ganglion located on the rostral right lateral aspect of the conus trunci and is found in small groups of four to five cell clusters embedded in fatty connective tissue. The IVS ganglion is the largest ventricular ganglion. It is found deeply embedded in the ventricular myocardium at the posterior aspect of the interventricular septum. This ganglion is composed of large round clusters of cells surrounded by fatty connective tissue. Individual clusters typically contain 40–60 cells. This ganglion begins at the rostral aspect of the ventricular myocardium and ends just below the aortic outlet. The LV2 ganglion is a smaller ventricular ganglion found on the epicardial surface of the left ventricle in small elongated and somewhat scattered clusters embedded in fat tissue and often in close association with epicardial blood vessels. Although PGP 9.5 immunoreactivity permitted the initial mapping of the locations of intracardiac ganglia, actual counts of both labeled and unlabeled neurons within these ganglia (Fig. 4) were made at ×200 magnification using fluorescence microscopy with a FITC excitation and barrier filter set (Nikon) because this optical system permitted the ready differentiation of neuronal cytoplasm, nuclei, and axons from other tissue elements within the heart. Nuclear labeling of retrogradely labeled neurons with diamidino yellow was also readily observed with this optical system but was confirmed by using a UV optical filter that visualizes diamidino yellow (Nikon). By contrast, it was often difficult to estimate cell counts with PGP 9.5 because the nuclei of PGP 9.5-immunoreactive neurons as well as the cytoplasm are both strongly labeled. Therefore, the presence or absence of nuclear boundaries within a cell could not be used to help differentiate neurons from fragments. Other cardiac landmarks were also poorly distinguished in these tissues as well. While examining adjacent control sections, however, we observed that by using FITC optics we could much more clearly distinguish and count neurons within intracardiac ganglia on the basis of their mor-
Percent labeling in all other ganglia was not significantly different from one another ($P > 0.16$) and averaged $6.51 \pm 2.54\%$. Note particularly that very few retrogradely labeled neurons were found in the CV ganglion (Figs. 5 and 6).

Subsequent to multiple microinjections of diamidino yellow into the anterior apical surface of the left ventricle (Fig. 2), there was a statistically significant difference in the percentage of the total number of neurons that were retrogradely labeled within individual intracardiac ganglia [F(7,22) = 16.76, $P < 0.001$; Fig. 7]. In this case, more than three-fourths of the retrogradely labeled neurons found in the heart were located in three ventricular ganglia, i.e., the CV, IVS, and LV2 ganglia. Percent labeling in the IVS ganglion was significantly larger than that observed in any other intracardiac ganglion (mean = 42.8 \pm 7.6\%; $P < 0.05$). There was no significant difference in retrograde labeling observed between the CV and LV2 ganglia (mean = 18.4 \pm 3.2\%; $P > 0.05$); however, labeling in the CV ganglion was significantly greater than that observed in the SA, AV, IAS, or RV ganglia ($P < 0.05$). Percent labeling in all ganglia other than the CV, IVS, and LV2 ganglia averaged 4.8 \pm 0.93\%. When diamidino yellow was injected into the pericardium in the control animal, 49 \pm 0.54\% of all neurons within each intracardiac ganglion were labeled.

**DISCUSSION**

Considerable uncertainty exists concerning the anatomic location and precise physiological functions of the intracardiac ganglia of multiple species, including the cat. Because the sympathetic input to the heart largely arises from neurons located in the intrathoracic ganglia (3), intracardiac ganglia are believed to be predominantly parasympathetic, although a few neurons containing catecholamines have been found on atrial ganglia (19). Some evidence also indicates that intracardiac ganglia may contain sensory neurons and local circuit neurons (2). Although it has been long known that vagal efferents to the heart richly innervated parasympathetic ganglia located on the atria, it was generally believed that vagal parasympathetic control either did not extend to the ventricles or had minimal physiological relevance (29). In the cat, Calaresu and St. Louis (10) reported that intracardiac ganglia were found surrounding...
the great vessels and on the atria, or within the interatrial septum. However, “ganglion cells were never seen in the kitten ventricles” (Ref. 10; p. 61). This conclusion was based, however, on an examination of the ventricles in only one kitten. In analogy with the data in the cat, early data suggested that the rat and dog ventricles also contained few or no intracardiac ganglia (8, 11). Recent evidence, however, indicates that at least one intraventricular ganglion is present in both the cat (20) and the rat heart (34, 35), and multiple intraventricular ganglia are present in the dog heart (39). In the present report, therefore, we have examined sections throughout the atria and ventricles of the cat heart for the presence of intracardiac ganglia. Our data indicate that there are eight regions of the cat heart that consistently contain ganglia. Within the atria, we have previously defined the location of two intracardiac ganglia, i.e., the SA and AV ganglia (21). We have now determined the distribution of two additional atrial ganglia, i.e., the PA ganglion and the IAS ganglion. Furthermore, we have detected a total of four intraventricular ganglia, one on the right ventricle, two on the left ventricle, and one within the interventricular septum. Although there is some homology between species in the distribution of intracardiac ganglia, there are also substantial interspecies differences in the number of atrial or ventricular ganglia, their distribution, and in estimates of the total number of neurons found in the heart (5, 6, 10, 27).

The distribution of parasympathetic postganglionic nerves to many regions of the heart, including both ventricles, has largely been inferred from physiological studies (1, 8, 12, 29), not neuroanatomic studies. We have previously determined the intracardiac location and physiological effects of the CV ganglion on cardiac functions. It was reported that microinjections of a ganglionic blocking drug into the CV ganglion attenuated the negative inotropic effects of vagal stimulation on the left ventricle, without influencing either vagally mediated negative chronotropic or negative dromotropic effects (18, 20). These physiological data predict that, at a minimum, neurons in the CV ganglion should project to the left ventricle and not to other regions of the heart. In the present study, a retrograde tracer was placed into the anterior apical surface of the left ventricle because some data indicate that the autonomic innervation of the ventricles is not homogeneous. Thus, for example, the sympathetic postganglionic innervation of the anterior apical surface of the left ventricle arises from the left stellate ganglion, while the corresponding innervation of the posterobasal wall arises from the right stellate ganglion (30). We observed that the CV ganglion was robustly labeled subsequent to microinjections of tracer into the left ventricle but was not significantly labeled subsequent to tracer injections into the right ventricle. These neuroanatomic data are therefore consistent with our previous physiological hypothesis that the CV ganglia.
ganglion selectively mediates a negative inotropic effect on the left ventricle because the CV ganglion does not effect chronotropic or dromotropic cardiac functions (18, 20) and does not project to the right ventricle to potentially influence the physiological function of this chamber. The present data further indicate, however, that although the physiological effects mediated by the CV ganglion are selective, it is not the exclusive source of vagal efferents to the left ventricle. Rather, two other intraventricular ganglia also significantly mediate vagal control of left ventricular functions. These include the IVS and LV2 ganglia. Percent labeling in the IVS ganglion was significantly larger than that found in any other intracardiac ganglion, and more than three-fourths of the retrogradely labeled neurons found in the heart were located in these three ventricular ganglia. By contrast, the percent labeling in each of the other ganglia averaged <4%. Further studies will be required to determine the precise physiological roles of the IVS and LV2 ganglia in the control of left ventricular functions and the potential extent of their influence on left ventricular contractility.

To control for potential labeling due to leakage of tracer from the injection sites, tracer was injected into the pericardial space. This resulted in a uniform 49 ± 0.54% degree of neuronal labeling within each intracardiac ganglion. By contrast, injections of tracers into either the left or right ventricular wall resulted in a highly statistically significant difference (P < 0.001) in percent labeling across the different intracardiac ganglia. We conclude from these data that leakage of the tracer from its injection sites was minimal. We have also considered the possibility that the degree of retrograde ganglionic labeling seen with diamidino yellow might potentially be proportional to the distance between labeled ganglia and the sites of injection of this tracer. In this way, relatively close ganglia would be labeled, whereas distant ganglia would not. Several lines of evidence indicate that failure to find retrogradely labeled neurons within various intracardiac ganglia subsequent to injections of the tracer into either ventricle was not due to relative distance from the injection site or inadequate survival time. Thus, for example, the present data indicate that injections of tracer into the right ventricle failed to label neurons in a closely adjacent ganglion actually contained within the right ventricle (the RV ganglion) while significantly labeling neurons approximately equidistant from the injection sites in the interventricular septum (the IVS ganglion). Furthermore, the 4-day survival time that was used in the present report has also been demonstrated to provide ample staining of cardioinhibitory vagal preganglionic neurons in the nucleus ambiguus of the brain stem of the cat after microinjections into the SA or AV ganglia (7), a distance that is substantially greater than that separating all intracardiac ganglia from injection sites in either the left or right ventricle.

The present data indicate that the vagal postganglionic control of right ventricular function is mediated primarily from the IVS ganglion, which contained more than two-thirds of the retrogradely labeled neurons found in the heart. This was statistically significantly greater than that of any other intracardiac ganglion. By comparison, average percent labeling in all other ganglia was not significantly different from one another and averaged ~6% of the total labeling in the heart. The IVS ganglion, therefore, is the largest source of neurons innervating the anterior surfaces of both the right ventricle and the left ventricle. It is interesting to speculate that this dual-innervation pattern may help to coordinate or segregate vagal effects on ventricular performance. It would be interesting indeed to inject two separate retrograde tracers into the left and right ventricles to see whether the same neurons in the IVS ganglion project to both targets or whether separate populations of neurons within this ganglion project to individual ventricles.

Greater understanding of the pathways and mechanisms whereby the vagus nerve exerts its effects on the ventricles may be helpful in understanding autonomic responses during acute myocardial ischemia. Autonomic responses during acute myocardial ischemia play a major role in predicting the probability of a fatal outcome. If activation of the sympathetic nervous system occurs, malignant ventricular fibrillation and sudden death are likely (31). If, on the other hand, acute myocardial ischemia is followed by activation of cardiac vagal efferents, a protective antifibrillatory effect is seen and the probability of survival is enhanced (16, 37). These results may be due to the fact that enhanced vagal tone increases ventricular fibrillation threshold and reduces the arrhythmogenic effects of sympathetic activation (37). Human studies indicate that vagal activity is reduced for several months after myocardial infarction, whereas sympathetic tone is enhanced (32, 34, 48). It is believed that this altered autonomic balance may in part mediate the increased incidence of sudden cardiac death after myocardial infarction (26). The autonomic nervous system also has a profound effect on the pathogenesis of numerous arrhythmias, including supraventricular tachycardias, atrioventricular block, and ventricular arrhythmias associated with myocardial ischemia and sudden death (13, 26). These data emphasize the clinical importance of understanding how the parasympathetic nervous system regulates ventricular functions. This knowledge is essential to the future rational design of experimental interventions in heart disease.

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