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The Relative Density of Sympathetic Nerve Terminals in the Canine Right Atrium[1] (39619)

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Although numerous studies have been made of the regional differences in sympathetic nerve supply to the heart of the dog, technical limitations have prevented quantitative estimates of the density of nerve endings within small portions of a given chamber. Chemical assays for norepinephrine, histochemical fluorescence, and radioactive tracer techniques have furnished important information, but definitive descriptions of precise distributions of sympathetic terminals are still lacking. As pointed out by Angelakos (1) in 1965, no samples from small portions can be representative of the whole heart, nor can estimates from the innervation of the whole heart accurately reflect distribution to selected regions. Even the specific question of the efferent innervation of the SA nodal region remains open at the present time. To obtain answers to the important questions of specific innervation of selected regions of the right atrium, the entire chamber was examined under neurally intact and extrinsically denervated states by a radioactive tracer technique.

Methods. The studies employed the uptake of tritiated norepinephrine to map the relative densities of sympathetic nerve terminals in 20 consecutive sections of the right atrium, including the interatrial septum. Eight of the 18 dogs used were subjected to total cardiac denervation (2) approximately 2 weeks prior to the uptake study. The aseptic, surgical procedures for denervation included careful dissection around the complete circumference of the superior vena cava (SVC), transection of the azygous vein, and dissection around the right pulmonary artery. The dissection was continued medially from the SVC across the superior surface of the right and left atria, and the adventitia was removed from the complete circumference of the pulmonary artery. All nervous tissue was also removed between the roots of the pulmonary artery and the aorta. The ventrolateral cervical cardiac nerve was transected and all nervous tissue was removed from the region around the left superior pulmonary vein. Inotropic and chronotropic responses to bilateral stimulation of the thoracic vagosympathetic trunks, together with the left stellate ganglion, were performed immediately following surgery, and completeness of denervation was certified. These tests were repeated at the time of sacrifice in order to confirm the denervation.

Under phencyclidine HCl (2.0 mg/kg) and alpha chloralose (80 mg/kg) anesthesia, thoracotomized dogs were administered a slow injection containing 5 μCi of [1-^{3}H]norepinephrine (Amersham-Searle) via the SVC. After 20 min, the heart was excised and the right atrium and interatrial septum were dissected and consistently sectioned into 20 small portions of about 250-mg each, and immediately were immersed in cold saline (0.9% NaCl). The appendage was cut along its fold from its ventricular border to the SVC so that the entire chamber could be laid out flat. Natural landmarks such as the fossa ovalis, coronary sinus, and the crista terminalis allowed consistent separation into the 20 sections mapped in the upper portion of Fig. 1. The tip of the appendage is comprised of sections 1, 2, 3, 19, and 20. The interatrial septum was included in sections 13 and 14 and the SA node was always in section 5. All visible fat was trimmed from the sections, and the atrium was frozen before preparation for liquid scintillation counting.

Thawed sections of right atrium were weighed and ground in a Waring blendor with microattachment in 5 ml of 0.4 *N* perchloric acid. After grinding for 3 min, each section was centrifuged at 4000*g* for 10 min

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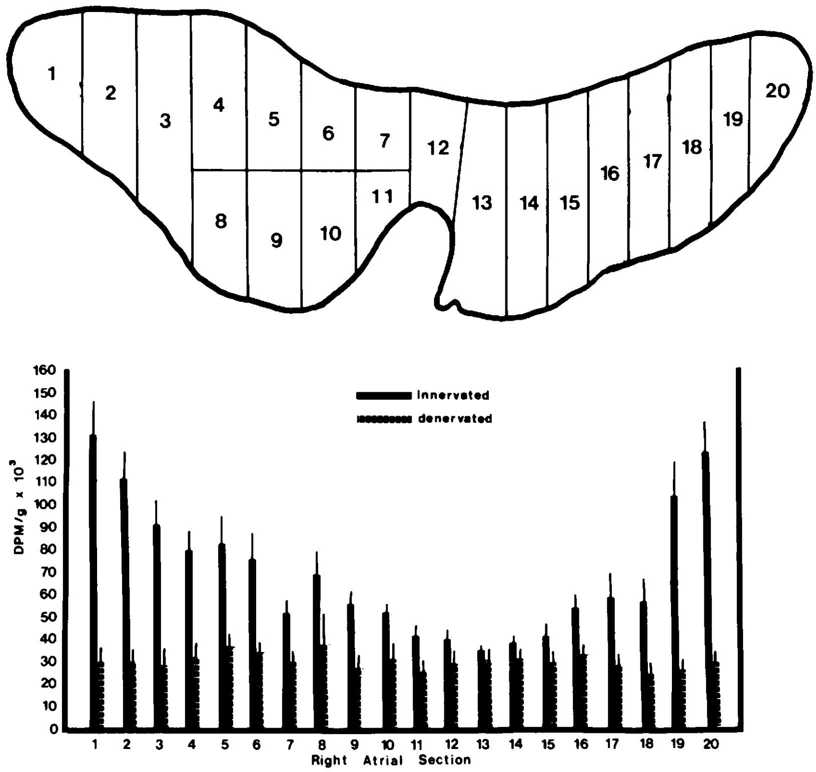

FIG. 1. The upper portion illustrates the sectional layout of the right atrium as observed from the endocardial surface. Sections 1 and 20 represent the apex of the appendage. The interatrial septum includes sections 13 and 14. The anterior free wall includes sections 4 through 11 and the posterior free wall sections 15 through 18. Lower portion illustrates the distribution of radioactivity (disintegrations per minute per gram $\times 10^3$) in innervated (solid bars) and totally denervated (striped bars) dog hearts following intravenous tritiated norepinephrine. Thin lines represent standard deviation.

in an International portable refrigerated centrifuge. Duplicate 1.0-ml samples of the supernatant were added to 9 ml of phase-combining solution (PCS, Amersham-Searle) which served as both a solubilizer and a scintillation cocktail. The samples were counted by liquid scintillation in a Searle-Analytic Isocap 300 System. The sample channels ratio method of quench correction was applied to each counting period and background radioactivity was taken into account. Uptake was then computed as disintegrations per minute of tritiated norepinephrine per gram wet weight of atrial tissue. All data were analyzed for statistical significance using one-way analysis of variance followed by a Scheffe test for multiple group comparisons.

Results. Figure 1 (lower portion) shows mean disintegrations per minute per gram of atrium (dpm/g) for each section of neurally intact atrium (solid bars) with standard errors of the mean (narrow bars). In general, a gradient of radioactivity became apparent from a high concentration in either tip of the appendage (section 1 = 130,000 dpm/g, section 20 = 122,000 dpm/g) to a low in the interatrial septum (section 13 at 35,000 dpm/g and section 14 at 37,000 dpm/g). Section 5, containing the SA node at 85,000 dpm/g was intermediate in general level of radioactivity. Statistical analysis showed section 1 to be significantly more radioactive than 7 ($P < 0.005$), 9–11 ($P < 0.005$), 12–15 ($P < 0.001$), 16–17 ($P < 0.025$), and 18 ($P < 0.01$). Section 2 was higher than 11 ($P < 0.05$), 13–14 ($P < 0.025$), and 15 ($P < 0.05$). Section 20 was higher than 11 ($P < 0.005$), 12 ($P < 0.01$), 13–14 ($P < 0.001$), and 15 ($P < 0.005$). No other comparisons were statistically different.

Figure 1 also shows mean disintegrations per minute per gram for each segment of the previously denervated heart (striped bars). In general there was no gradient of radioactivity between the regions of the totally, extrinsically denervated right atrium. Statistical analysis revealed no significant differences between any two sections. The average radioactivity of all 20 sections was 28,000 dpm/g. Compared to 130,000 dpm/g for section 1 of the innervated atrium, this represents a reduction to 21.5%.

Discussion. Other studies employing different techniques have investigated the regional differences in sympathetic supply to the dog heart and have reported varying results and conflicting conclusions (3–7). However, the concept of high levels of radioactivity in the appendage, implying a region of dense sympathetic nerve terminals, has support in the literature. Dahlstrom (4) found a "large number" of sympathetic terminals there although she offered no quantitative description. The significance of this high density may be functional in regulating a locale of increased contractility required during the stress of acute distention. This hypothesis requires further study.

Histochemical fluorescence techniques have demonstrated marked variability in distribution of adrenergic innervation to atrial tissues. Fluorescence patterns have indicated a rich supply to atrial tissues (as compared to ventricular) with a particularly dense supply to SA nodal regions (4). The supply to Purkinje cells, on the other hand, is said to be very sparse, with many such cells receiving no adrenergic innervation at all. Finally, these histochemical techniques have clearly differentiated rich distribution of adrenergic nerves to "upper regions" of the right atria from that to areas near the AV node which are relatively poorly supplied (3, 4). Considerable functional importance could be imputed to a particularly dense distribution of adrenergic fibers to the specialized internodal conduction pathways, but neither the fluorescence nor the present tritiated norepinephrine uptake studies lend credence to that mechanism.

On the other hand, there is conflicting evidence concerning the densities of terminals within and around the SA node. Shindler (6) reported a higher concentration of endogenous norepinephrine in this location than in the remainder of the right atrium. Serrano (5), however, concluded from measurements of endogenous norepinephrine that the SA node did not have higher levels. Shindler compared the SA node to atrium exclusive of the appendage, and in light of the present data, this would account for the difference. However, extrapolation from SA nodal region concentration does not necessarily prove that the SA node itself is not well supplied with sympathetic nerves, because it is a relatively small portion of the total tissue sample. Based upon calculations of SA nodal volume of 5.0 mm^3 (8), the SA node represents only about 5% of the volume of the total sample (approximately 100 mm^3).

The result of a statistically significant peak of radioactivity in the right atrial appendage attests to the sensitivity of $[^3H]$norepinephrine uptake as a quantitative evaluation of sympathetic nerve terminal density. The results uphold the expectation that this technique is a useful gauge for the status of denervation. The chronically denervated sections were, on the average, 21.5% of the maximum levels observed in the innervated atria. The fact that uptake was not reduced to zero after denervation was to be expected on the basis of known extraneuronal uptake mechanisms (9). The disappearance from denervated atria of the gradient of radioactivity seen in neurally intact atria confirms the assumption that the gradient was produced by the presence of sympathetic nerves, and that lack of that gradient indicates sympathetic denervation.

Summary. Relative densities of sympathetic nerve terminals in 20 consecutive sections of both normally innervated ($n = 10$) and chronically denervated ($n = 8$) dog right atria were determined by comparative uptakes of $[^3H]$norepinephrine. Results of analysis of neurally intact atria display a consistent gradient of $[^3H]$norepinephrine with the highest concentration (130,000 dpm/g) in the tip of the atrial appendage and the lowest (30,000 dpm/g) in the intera-

trial septum. The region containing the SA node was not distinguished by significantly high uptake. Atria of chronically denervated hearts had a markedly diminished [^{3}H]norepinephrine uptake (average of 28,000 dpm/g). No gradient of radioactivity between sections was present in the denervated atria. These results indicate that (1) the canine right atrium has marked concentrations of sympathetic terminals in the appendage, (2) the technique of [^{3}H]-norepinephrine uptake gives a quantitative evaluation of sympathetic nerve terminal density, and (3) the technique allows for evaluation of the status of cardiac denervation.

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