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Regional Uptake of [^{3}H]Norepinephrine by the Canine Left Ventricle[1] (41491)

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Abstract. The distribution of sympathetic neurons within the canine left ventricle was assessed by measuring the myocardial uptake of [^{3}H]norepinephrine in anesthetized dogs. The nonneuronal uptake of [^{3}H]norepinephrine was also assessed in a separate group of cocaine-treated animals. The left ventricle was systematically divided into multiple sections. The [^{3}H]norepinephrine was isolated in tissue homogenates, using alumina extraction, and quantified by liquid scintillation counting. The results revealed a nonuniform pattern of [^{3}H]norepinephrine uptake in the ventricular free wall, with a greater uptake in the base than in the apex. Differences in uptake between the anterior and posterior regions of the free wall, and between the free wall and the septum were not statistically significant. In the cocaine-treated animals uptake was approximately 20% of that in the control animals. Furthermore, there were no significant regional differences. These data suggest that in the canine left ventricle the sympathetic nerves are distributed nonuniformly between the base and apex, but otherwise the distribution is uniform.

Although neural control of the heart is a topic of considerable current interest (1, 2), very little information is available on the regional distribution of cardiac sympathetic neurons within the left ventricular myocardium. The sympathetic nerve supply to the left ventricle has been shown to cross from the pulmonary artery-aortic region to the ventricle and to course in the epicardium in a base to apex direction before branching and innervating the deeper layers of the myocardium (3–5). In previous studies concerned with regional innervation of the canine left ventricle, Angelakos (6) found a difference in norepinephrine content between the base and the apex, and Dahlström *et al.* (7) obtained histological evidence of nonuniform transmural sympathetic innervation.

The purpose of the present study was to characterize further the regional sympathetic innervation of the canine left ventricle. To accomplish this we utilized the [^{3}H]norepinephrine uptake method of Kaye and Tyce (8), in which [^{3}H]norepinephrine uptake has been shown to be a reliable index of cardiac sympathetic innervation. Results were obtained in both normal and cocaine-treated animals to distinguish between the neuronal and nonneuronal uptake of [^{3}H]norepinephrine in different regions of the ventricle.

Methods. The experiments were performed on 13 male mongrel dogs (20–40 kg) who had been maintained on a nourishing diet for at least 30 days. The animals were fasted overnight, premedicated with morphine sulfate (2.5 mg/kg, sc), and anesthetized 45–60 min later with α-chloralose (100 mg/kg, iv). Supplemental doses of α-chloralose were given as required prior to the administration of [^{3}H]norepinephrine, but additional anesthetic was avoided thereafter. The trachea was intubated with a cuffed endotracheal tube and the animal was ventilated with a Harvard respirator (Model 607). Supplemental oxygen was

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added to the inspired air to maintain a normal arterial oxygen tension as determined on an Instrumentation Laboratory blood-gas analyzer (113-S1). The right femoral artery and vein were isolated and catheterized, with the catheters advanced to the aortic arch and inferior vena cava. Arterial pressure was monitored with a Statham pressure transducer (P23Db) and an Electronics for Medicine (Model Dr-8) oscillograph. A left thoracotomy was performed through the fifth intercostal space, the pericardium incised, and the heart exposed. Gauze, damped in saline (0.9%), was placed on the left ventricular epicardium to prevent the epicardial surface from drying. Heparin (500 U/kg) was administered and a blood sample was taken for determination of arterial PO_2, PCO_2, and pH. Blood samples were analyzed immediately, and if required, ventilation was adjusted to maintain blood gases within normal ranges and sodium bicarbonate was given to maintain arterial pH above 7.30.

In eight animals 1-[7,8-^{3}H]norepinephrine (Amersham, 30–40 Ci/mmole) was administered (2.5 μCi/kg, iv) as described by Kaye and Tyce (8). The protocol involved the infusion of the [^{3}H]norepinephrine over a 5-min period, followed by a 20-min incubation period, which enabled the labeled norepinephrine to become incorporated into the neuronal pools and to clear the extracellular space. At the end of the 20 min the heart was rapidly excised and placed in chilled saline (0°). In five additional animals, cocaine was administered (iv) to block neuronal uptake, 330 μg/kg/min for 15 min prior to the [^{3}H]norepinephrine infusion and 66 μg/kg/min during the infusion and incubation periods (9).

Using a grid system, the left ventricle was divided into 33 sections in a cold room (25 transmural free wall sections and 8 septal sections) (Fig. 1). By utilizing the papillary muscles as landmarks it was possible to section the free wall into five "rows" from base to apex and five "columns" from anterior to posterior. All tissue samples were minced, weighed, and placed into tubes containing 0.4 *N* perchloric acid (0°). The minced sample was homogenized with two 10-sec bursts of a polytron. The homogenate was centrifuged at 10,000*g* for 20 min, the supernatant was decanted and frozen for later analysis. Two milliliters of the supernatant was placed into a tube containing 150 mg of alumina, which had been prepared according to the method of Anton and Sayre (10), and 3 ml of 2 *M* Tris–0.5 *M* EDTA buffer, pH 8.6 (at this pH norepinephrine is bound to the alumina, whereas the *o*-methylated metabolites are not). This solution was vortexed thoroughly, and the supernatant was aspirated. The alumina was washed three times with distilled water (2–3 ml) and the norepinephrine was eluted from the alumina by addition of 1 ml of 0.05 *N* perchloric acid and thorough vortexing. The acid was pipetted into a scintillation tube containing 10 ml of a toluene-based cocktail (3a20, Research Products International) and 3 ml of a detergent (Triton X-100 scintillation grade, Research Products International). Samples were counted for 10 min in a Packard Tri-Carb liquid scintillation spectrometer. Quenching standards were used to calculate efficiency which averaged 44%. [^{3}H]Norepinephrine standards were used to calculate the percentage yield of the alumina extraction procedures, which averaged 41%. All sample counts were corrected for tissue weight, efficiency, percentage yield, and counting time, yielding results expressed as disintegrations per minute per gram (dpm/g). When duplicate [^{3}H]norepinephrine uptake values were compared, the coefficient of variation (r^2) was 0.95.

Differences among the means of the 33 sections for each of the two groups (control and cocaine-treated) were analyzed by analysis of variance. If the *F* value was significant ($P < 0.05$), a further statistical analysis was performed on the 25 free wall sections as follows: the data for individual sections were combined into 5 horizontal "rows" for making comparisons between the base and apex and parallel intermediate regions and into 5 vertical "columns" for making comparisons between the anterior and posterior regions and parallel intermediate regions, using analysis of variance and multiple comparison testing.

Results. The results obtained in the eight control animals are shown in Figs. 1–3. Shown in Fig. 1 are the [³H]norepinephrine uptake data for all 33 tissue sections. Analysis of variance of the 33 sections was significant ($P < 0.05$). Since this indicated that the uptake of [³H]norepinephrine was nonuniform, further statistical testing was performed. Shown in Fig. 2 are the [³H]norepinephrine uptake data for the larger regions of the left ventricular free wall obtained by combining sections into "rows." Analysis of variance was significant ($P < 0.05$), indicating that [³H]norepinephrine uptake was nonuniform in a base to apex direction. Further analysis revealed that uptake was significantly greater in the two most basilar "rows" than in the apical "row." Uptake in the two intermediate "rows" adjacent to the apex was not significantly different from that in the apical "row" or the two most basilar "rows."

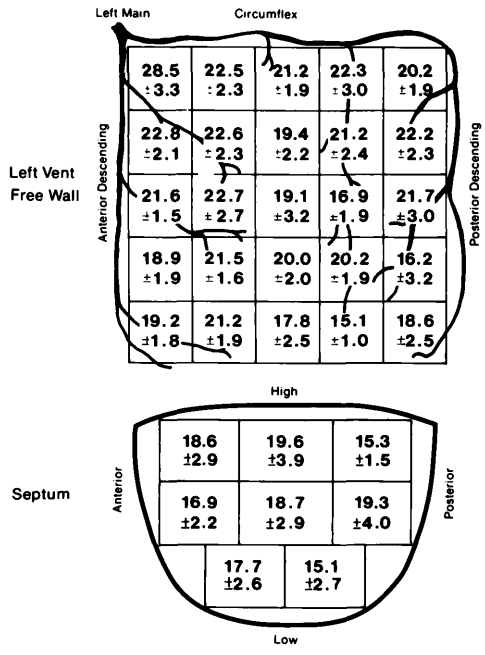

FIG. 1. [³H]Norepinephrine uptake in 25 regions of the left ventricular free wall and 8 regions of the interventricular septum obtained by systematically sectioning the ventricle into 33 tissue samples. The values shown, multiplied by 10^3, represent disintegrations per minute per gram. They are the means and standard errors for eight control animals.

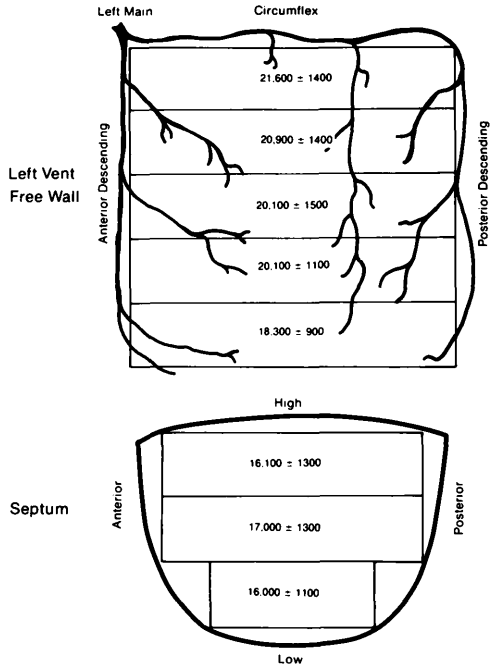

FIG. 2. Myocardial [³H]norepinephrine uptake data (dpm/g) as analyzed in a base to apex direction. Values are mean ± SEM.

Shown in Fig. 3 are the [³H]norepinephrine uptake data for the larger regions of the left ventricular free wall obtained by combining sections into "columns." Analysis of variance was insignificant ($P > 0.1$), indicating that [³H]norepinephrine uptake was uniform in an anterior to posterior direction. Analysis of variance of the eight sections of the septum was insignificant ($P > 0.05$), indicating that [³H]norepinephrine uptake was uniform in the septum.

The average [³H]norepinephrine uptake value for the entire left ventricular free wall was 20,000 ± 1200 dpm/g, whereas that for the septum was 16,000 ± 2000 dpm/g. The difference was not statistically significant ($P > 0.1$).

Results obtained in the cocaine-treated animals are shown in Fig. 4. Depicted are the [³H]norepinephrine uptake data for all 33 tissue sections. Analysis of variance of the 33 sections was insignificant ($P > 0.1$), indicating that nonneuronal [³H]norepinephrine uptake in the left ventricular free wall and septum was uniform. The average

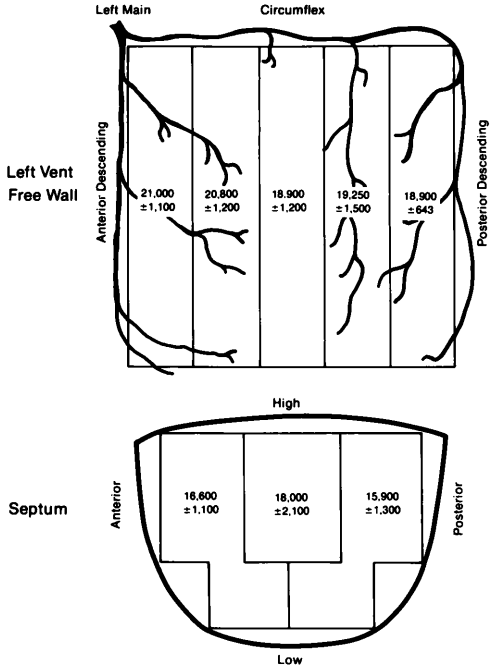

FIG. 3. Myocardial [³H]norepinephrine uptake data (dpm/g) as analyzed in an anterior to posterior ventricular wall direction. Values are mean ± SEM.

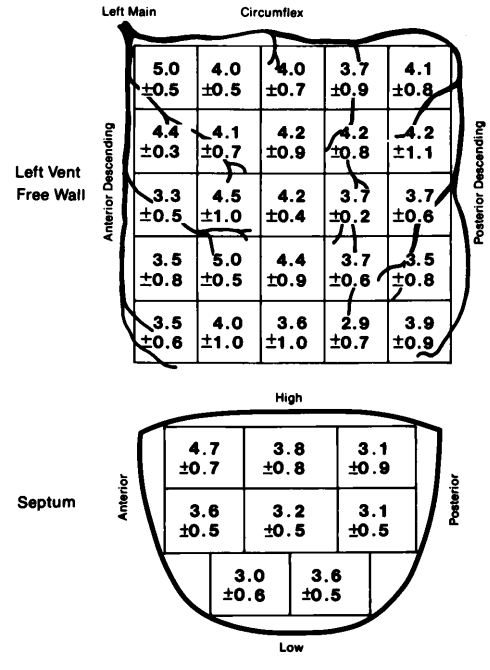

FIG. 4. [³H]Norepinephrine uptake portrayed as in Fig. 1, but for five cocaine-treated animals.

[³H]norepinephrine uptake value for the entire left ventricular free wall was 4000 ± 500 dpm/g, whereas that for the septum was 3500 ± 500 dpm/g. [³H]Norepinephrine uptake for the free wall and septum in the cocaine-treated animals was significantly less than that in control animals, with the values averaging approximately 20% of those obtained in the control animals.

Discussion. The major findings of this study are: (i) that a regional difference in neuronal [³H]norepinephrine uptake was observed between the base and apex of the ventricular free wall, with the uptake being approximately 20% greater in the base than in the apex, and (ii) nonneuronal [³H]norepinephrine uptake is uniform throughout the left ventricle, and it amounts to approximately 20% of that taken up by the normal ventricle.

The method of [³H]norepinephrine uptake to assess cardiac sympathetic efferent innervation has been extensively utilized by several laboratories (8, 11, 16). [³H]Norepinephrine uptake was reported to be a more reliable indicator of sympathetic innervation than norepinephrine content of the heart (8). Alumina extraction of norepinephrine eliminates methylated metabolites, which have the tritium label, but not the deaminated metabolites. However, in the heart the deaminated metabolites are reported to constitute only 1–2% of the recovered label after tracer norepinephrine uptake studies (17). The recovery of norepinephrine in this study was 41%, which is lower than that usually reported in the literature. However, the recovery was consistent among the experiments and the coefficient of variation of the procedure was 0.95. When the absolute uptake values were normalized for the dose of tracer norepinephrine, and compared to those of Kaye and Tyce (8), also normalized for the dose of tracer norepinephrine, the values were virtually identical. Thus, we believe our results reflect the uptake of [³H]norepinephrine and the pattern of sympathetic innervation.

In the canine left ventricle, the sympathetic innervation has been termed

"patchy" (7) and two earlier studies suggested that innervation is greater in the base than in the apex (6, 7). Evidence that the catecholamine content of the base is higher than that of the apex has also been provided in a more recent study (8). However, in that study, no difference of [^{3}H]-norepinephrine uptake was found between the base and apex. The reason for the difference in findings between that study and the present study is not clear. One possible explanation is a more discrete separation of the ventricular free wall into apical and basilar regions in the present study. Only the [^{3}H]norepinephrine uptake value for the apical "row" was significantly different from that for the most basilar "rows," whereas the value for the intermediate "row" immediately adjacent to the apical "row" was not significantly different from the two most basilar "rows." Others have demonstrated that the sympathetic efferent innervation courses in the epicardium from the anterior basilar region to the apical and posterior regions (3). In addition, it has been shown that when the canine heart is surgically denervated, reinnervation returns in a base to apex sequence (11). The present results support the notion of a greater sympathetic innervation in the base than in the apex. The [^{3}H]norepinephrine uptake was approximately 20% greater in the basilar region than in the apical region.

One reason for regional variations in sympathetic innervation of the ventricle could be an association of the sympathetic nerves with the coronary vasculature. It has been shown that sympathetic nerves travel in the adventitia of the coronary conductance vessels before innervation of the myocardium (12, 13). We have shown in another study (14) that by applying phenol to selective sites on the myocardium and epicardial conductance vessels it is possible to produce a regional sympathectomy of the left ventricle.

In cocaine-treated animals the [^{3}H]norepinephrine uptake was uniform throughout the ventricle. These results favor the conclusion that the greater basilar [^{3}H]-norepinephrine uptake in the normal animal was due to a regional difference in sympathetic innervation rather than to a regional difference in myocardial blood flow or some other experimental variable. More direct evidence against a regional difference in blood flow has also been obtained in other studies on this animal preparation, using the microsphere method to measure blood flow. The nonneuronal uptake sites consist of myocytes, vascular smooth muscle, connective tissue and fibroblasts (15), which are distributed homogeneously in the myocardium.

The physiological significance of a nonuniform distribution of sympathetic nerves between the base and apex of the ventricular wall is not elucidated by the present study. Others have demonstrated localized changes in myocardial function by stimulating discrete branches of the cardiac sympathetic nerve supply (2) which suggests that overall ventricular function may be modulated by regional differences in cardiac sympathetic nerve function.

In conclusion, the present study provides further evidence of a nonuniform distribution of sympathetic neurons between the base and apex of the canine left ventricle, with a greater density of neurons in the base than in the apex. The regional distribution of sympathetic neurons otherwise appears to be uniform in the left ventricular free wall and septum. The study also indicates that the nonneuronal uptake of norepinephrine is uniform in the left ventricle, and the nonneuronal uptake constitutes approximately 20% of labeled norepinephrine uptake.

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