Depletion of Ventricular Catecholamine Levels Following Peripulmonary Neurectomy[1] (35156)

MICHAEL P. KAYE, DONALD V. PRIOLA, AND JOHN COYLE

Department of Physiology and Surgery, Loyola University, Stritch School of Medicine, Maywood, Illinois 60153; and the Department of Pharmacology, University of New Mexico School of Medicine, Albuquerque, New Mexico 87106

Recent studies in our laboratories have offered functional evidence that large numbers of sympathetic fibers reach the dog heart via the peripulmonary route and the ventrolateral cervical cardiac nerve (1). Histological studies of Cooper and co-workers (2) in cats have also indicated that these routes provide a rich source of cardiac sympathetic nerve fibers.

The present study was undertaken to determine the effects of surgical dissection of the peripulmonary tissues and/or transection of the ventrolateral cervical cardiac nerve on catecholamine levels in specific areas of the canine myocardium. In this manner we hoped to determine: (i) the relative quantity of sympathetic, postganglionic fibers reaching the heart via these routes; and (ii) the extent to which these two pathways provide innervation to the atria, ventricles, and interventricular septum.

Methods. Thirty mongrel dogs were placed into three equal groups. In Group I, the ventrolateral cervical cardiac nerve [VLCCN, as designated by Mizeres (3)] was transected at the junction of the pulmonary veins and left atrium. Animals in Group II underwent a peripulmonary dissection procedure (PPD) which included transection of the tissues surrounding the main pulmonary artery (including the adventitia) approximately 1 cm distal to the pulmonary valve annulus. In Group III, animals were subjected to both procedures. The animals were anesthetized with sodium pentobarbital (32 mg/kg) after which dissection of the cardiac sympathetic nerves was performed through a left thoractomy under aseptic conditions. The animals were sacrificed 2 weeks following surgery by rapid intravenous injection of sodium pentobarbital. The hearts were immediately removed, rinsed in cold saline, and sectioned. Representative samples of myocardium were taken from the right atrium (RA), the left atrium (LA), interventricular septum (IVS), right ventricular base (RVB), right ventricular apex (RVA), left ventricular base (LVB) and left ventricular apex (LVA). The samples were immediately frozen in liquid nitrogen and stored in a freezer at −10°.

Analysis for norepinephrine (NE), epinephrine (EPI) and total catecholamine content was subsequently performed using a trihydroxy indole method (4). The method was modified to obtain the maximum amount of catecholamine from the tissue. In the calculation of catecholamine content, a correction factor was introduced to adjust for loss of catecholamine during the recovery procedure. This factor was based upon the percentage recovery from a sample of known catecholamine concentration which was analyzed concurrently with each one of the tissue samples. Total catecholamine levels were obtained using fluoresence readings of aliquots at a pH of 6.5 employing an activation wavelength of 395 mμ and an emission wavelength of 500 mμ. Similarly, epinephrine levels were obtained at pH 3.5, utilizing an activation wavelength of 410 mμ and an emission wavelength of 515 mμ. Norepinephrine concentration was calculated as the total catecholamine minus the epinephrine. The results were expressed as micrograms of catecholamine per gram of wet weight. All data were analyzed using an unpaired *t* test

[1] Supported by Grant Nos. HE-08682 and HE-10869 from the National Institutes of Health and the Otho S.A. Sprague Foundation.

TABLE I. Catecholamine Levels (μg/g of wet wt) in Unoperated Control Hearts ($n = 6$).

Region	Total catecholamine ± SE	Epinephrine ± SE	Norepinephrine ± SE
Right atrium	2.10 ± 0.17	0.10 ± 0.01	2.00 ± 0.16
Left atrium	2.00 ± 0.17	0.10 ± 0.01	1.90 ± 0.16
Interventricular septum	0.84 ± 0.08	0.06 ± 0.01	0.79 ± 0.08
RV apex	1.10 ± 0.23	0.07 ± 0.01	1.03 ± 0.22
base	1.07 ± 0.20	0.06 ± 0.01	1.00 ± 0.20
LV apex	0.72 ± 0.15	0.05 ± 0.01	0.66 ± 0.15
base	1.11 ± 0.15	0.07 ± 0.01	1.04 ± 0.15

in which differences between mean values were considered significant if the p value was $\leq .05$.

Results. Table I shows the values for total catecholamines, norepinephrine (NE) and epinephrine (EPI) obtained in the 7 areas sampled in six control hearts. Our values for catecholamine levels in these areas are somewhat higher, but otherwise agree very well with those reported in the literature (5) in terms of relative concentrations in the various areas of the heart. In general, NE content was greatest in the atria with levels in the RV and LVB of about 1 μg/g, with the LVA representing the lowest level of the seven areas routinely sampled. As expected, EPI levels in all areas studied were 0.1 μg/g or less. Because the predominant amine stored in sympathetic nerve terminals is considered to be NE, data for the effects of the denervation procedures are expressed exclusively in terms of NE levels.

NE contents of the 7 sampled areas of myocardium in the control group and the three experimental groups are illustrated in Table II. It is clear that PPD (II) or VLCCN section (I) or both (III) resulted in significant decreases of NE content in all areas. In the atria and IVS, PPD produced a significantly greater decrease in NE content than did VLCCN. However, in the RVA and both regions of the LV, there was no significant difference between the NE levels for Groups I and II. In the RVB, on the other hand, Group II animals exhibited significantly lower NE levels than did Group I.

In order to more clearly differentiate the effects of the three operative procedures on regional NE levels, the data have been expressed in terms of percentage of control NE

TABLE II. Norepinephrine Levels (μg/g of wet wt) in Control and Partially Denervated Hearts.[a]

Region	Control ± SE	Group I[d] ± SE	Group II ± SE	Group III ± SE
Right atrium	2.00 ± 0.16	0.93 ± 0.10	0.42 ± 0.11[b]	0.64 ± 0.15[b,c]
Left atrium	1.90 ± 0.16	0.59 ± 0.09	0.47 ± 0.13[b]	0.39 ± 0.09[b]
Interventricular septum	0.79 ± 0.08	0.33 ± 0.07	0.07 ± 0.04[b]	0.08 ± 0.04[b]
RV apex	1.03 ± 0.22	0.22 ± 0.04	0.14 ± 0.05	0.02 ± 0.01[b]
base	1.00 ± 0.20	0.36 ± 0.04	0.06 ± 0.03[b]	0.02 ± 0.01[b,c]
LV apex	0.66 ± 0.15	0.11 ± 0.04	0.10 ± 0.04	0.01 ± 0.003[b]
base	1.04 ± 0.15	0.17 ± 0.04	0.16 ± 0.04	0.02 ± 0.01[b]

[a] All values in operated groups are significantly different from control ($p \leq 0.001$).

[b] Significantly different from: Group I ($p \leq 0.05$); [c] Group II ($p \leq 0.05$).

[d] Group I, section of ventrolateral cervical cardiac nerve; Group II, dissection of peripulmonary region; Group III, combination of procedures employed in Groups I and II.

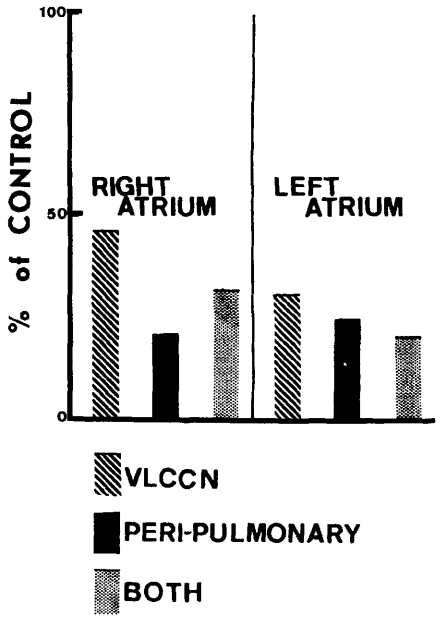

FIG. 1. Percentage of atrial norepinephrine remaining 2 weeks after section of the VLCCN and/or peripulmonary neurectomy.

content for the atria (Fig. 1) and the ventricles (Fig. 2). In both the RA and LA, PPD results in a decrease of NE content to 21 and 24.7% of control, respectively. In contrast, VLCCN causes decreases to 46.5% in the RA and 31% in the LA, values significantly higher than those produced by PPD. Performance of both procedures does not produce a significantly greater decrease in NE levels than PPD alone.

When the data for the ventricles are examined (Fig. 2), it is clear that PPD and VLCCN produce differential effects on RV and IVS, but not LV, catecholamine levels. In the septum, PPD alone produces a fall in NE content to 8.9% of control whereas VLCCN only results in a decrease to 41.8% of control. Performance of both procedures results in no greater decrease in NE levels than PPD alone. The situation is quite similar in the case of the RVB. Here, however, Group III animals exhibit a greater fall in NE content than that produced by either procedure alone. In the three other ventricular areas sampled (*i.e.*, RVA, LVB, LVA), either PPD or VLCCN produces a decrease of NE content to 13–21% of control. Performance of both procedures, on the other hand, results in a significantly greater decrease than either procedure alone; *i.e.*, to 2% of control.

Discussion. Functional studies from our laboratory (1) and histological work by others (2) have strongly suggested that a major portion of the cardiac sympathetic innervation reaches the heart via the VLCCN and the pulmonary artery. However, no information has been available on the relative quantities of sympathetic fibers which these pathways contribute to the heart and the relative patterns of distribution. If the assumption is made that myocardial catecholamine levels indicate, in a semiquantitative manner, the

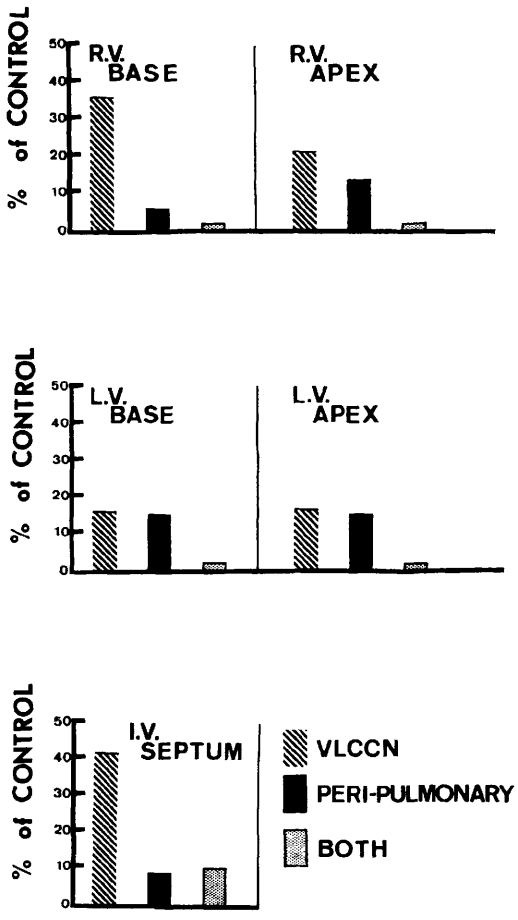

FIG. 2. Percentage of myocardial norepinephrine remaining 2 weeks after section of the VLCCN (ventrolateral cervical cardiac nerve) and/or peripulmonary neurectomy.

density of sympathetic postganglionic nerve endings in a given area of myocardium, the alterations which occur in regional NE levels should provide information on the quantity and distribution of nerves provided by the extirpated structures.

On the basis of the data presented, it is clear that dissection of the peripulmonary region combined with section of the VLCCN result in a fall of ventricular NE levels to less than 2% of control. This decrease is comparable to that produced by cardiac homotransplantation in which all nerve fibers supplying the heart are unquestionably severed (6) or by mediastinal neural ablation (7). These data strongly suggest that sympathetic postganglionic fibers traversing these two pathways provide virtually the entire innervation of the ventricles. Functional analyses provide firm support for this conclusion (1). Removal of both these pathways, however, causes a significant, but much lesser, decrease in atrial NE levels. Therefore, one would not expect the response of the atria to sympathetic nerve stimulation to be greatly impaired.

When the data on the regional effect of PPD are examined, the pattern of distribution of fibers traversing this pathway becomes clear. Although both pathways tested provide sympathetic fibers to the atria, a greater number appear to be provided via the peripulmonary route. PPD also causes a decrease in IVS catecholamine level which is equal to performance of both procedures (Group III). This strongly suggests that the majority of sympathetic innervation of the IVS is provided by fibers which reach the heart via the pulmonary artery. A much smaller, but still significant, number of fibers is provided to the IVS by the VLCCN. In addition, the RVB also appears to receive the majority of its sympathetic innervation via fibers traversing the peripulmonary region. The data also suggest this for the RVA, but the difference is not significant (Table II).

On the other hand, the LV appears to receive about the same number of fibers from both peripulmonary and VLCCN pathways. As shown in Fig. 2, performance of either procedure alone causes a decrease of NE content to approximately the same level. Only in Group III animals was there a decrease indicating that most, if not all, of the sympathetic supply had been abolished. This "cross over" innervation of the ventricles has been noted in other studies which utilized somewhat different techniques (8, 9).

Some of the ramifications of the information obtained in this study are obvious. If the canine data can be reasonably extended as being applicable to man, it is clear that dissection of the peripulmonary region or accidental damage to the equivalent of the VLCCN during surgical procedures may significantly impair the ability of the heart to respond to sympathetic neural modulation. For the basic scientist, the procedures utilized in Group III animals may provide a comparatively simple means of producing a heart with denervated ventricles. For example, this type of preparation might be extremely useful for studying the reactions of the cardiovascular system to neural stimulation in the absence of any significant contribution of the heart to the responses. It avoids the masive dissection called for in the mediastinal neural ablation procedure (7) and the imposing surgical expertise necessary for homotransplantation of the heart (6) while being capable of producing a preparation with equivalent ventricular denervation.

Summary. Norepinephrine levels were measured in seven different regions of canine hearts from four groups of animals: controls, animals subjected to section of the ventrolateral cervical cardiac nerve (Group I), animals in which the tissues surrounding the base of the pulmonary artery were dissected (Group II), and a fourth group in which both surgical procedures were done (Group III). Results of this study suggest the following conclusions: (i) The major sympathetic innervation of the ventricles is supplied by fibers which traverse the pulmonary artery and VLCCN; (ii) The major sympathetic innervation of the atria is via some pathway other than the pulmonary artery and VLCCN, although fibers coursing through the peripulmonary region supply a large number of fibers to the atrial myocardium; (iii) The interventricular septum and base of the right ventricle receive their major sympathetic innervation via fibers which pass through

the peripulmonary region; (iv) The left ventricle and right ventricular apex are supplied about equally with sympathetic fibers arriving via both pathways studied.

The authors acknowledge the technical assistance of Edith Higgins, Charles P. Montoya, and David H. Snape in the performance of the experiments.

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Received July 20, 1970. P.S.E.B.M., 1970, Vol. 135.