Chronic Extrinsic Cardiac Denervation by Regional Neural Ablation

Description of the Operation, Verification of the Denervation, and Its Effects on Myocardial Catecholamines

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The study of chronically denervated hearts for the purpose of isolating inherent myocardial reactions to given stresses, such as exercise,1-3 for study of the direct effects of drugs on the isolated myocardium,4-7 or in anticipation of the management of a transplanted heart, requires ideally that the denervation be complete and limited to the heart as far as possible, that it be tolerated chronically in a reasonably healthy animal, and that the completeness of the denervation be capable of verification.

Previous techniques for accomplishing chronic denervation in the dog have usually consisted of staged cervical or thoracic vagectomy and sympathetic ganglionectomy.1-7 Studies of epinephrine sensitivity performed in such preparations have yielded results which have been interpreted as showing both normal sensitivity and supersensitivity.5-7 From such experiments one also might erroneously conclude that the canine heart, since it is not totally depleted of catecholamines following sympathetic ganglionectomy,8 cannot be depleted of catecholamines by surgical denervation. An additional important consideration is the fact that division of these major trunks interrupts the distribution of autonomic nerves to other viscera and vascular beds and results in a preparation not identical to that resulting from transplantation of the heart. The cardiac response of such a preparation to stress such as exercise may certainly be modified by altered peripheral vascular reactions. A review of the pertinent neuroanatomy of the dog9-12 indicates that regional ablation of the autonomic structures in the mediastinum should result in an experimental preparation which circumvents the above objections. The surgical technique of this type of cardiac denervation and the verification of the result of the procedure and its effects on myocardial catecholamines are described in the present report.

Methods

Description of Operation

Adult mongrel dogs (10 to 25 Kg.) were anesthetized with intravenous thiopental sodium (30 mg./Kg.) and the lungs ventilated with 100 per cent O₂ by a demand-positive pressure respirator through a cuffed endotracheal tube. Digoxin, 0.015 to 0.030 mg./Kg., was administered intravenously following the induction of anesthesia as a prophylactic supportive measure.13 Femoral arterial pressure was monitored throughout the procedure.

A median sternotomy was made, and the phrenic nerves were freed from the pericardium and pleura beginning at the thoracic inlet and continuing to the diaphragm. The pericardium was opened from the diaphragm to its reflection from the great vessels at the base of the heart. The ascending aorta and the origins of the brachiocephalic and subclavian arteries were mobilized and stripped of adventitia. The superior vena cava was completely freed from the innominate vein to the right atrium, and the azygos vein was divided. By retracting the superior vena cava toward the right atrium and the aorta toward the left, the right pulmonary artery was exposed and dissected from its origin to its main branches (fig. 1). The fat, lymphatics, and nervous structures in the pretracheal region were excised, exposing the dorsal wall of the left atrium in this area. The ventral surface of the trachea was denuded from its bifurcation to the approximate...
Operative exposure of the mediastinal autonomic neural structures. The dissection of the deep cardiac plexuses in the right pretracheal region. Aorta (Ao.), left atrium (LA), trachea (Tr.), superior vena cava (SVC), right ventricle (RV), right pulmonary artery (RPA).

level of T3, excising all other mediastinal elements between the aorta and the superior vena cava. The left pulmonary artery was skeletonized from its origin to its major branches; the ligamentum arteriosum, the vestigial fold of Marshall, and the pericardial attachments were all denuded. By retracting the aortic arch cephalad and the left pulmonary artery caudad, the left atrial wall was exposed in the region of entrance of the left superior pulmonary vein, and the contiguous mediastinal tissue was excised (fig. 2). The pericardial reflections surrounding the remaining left pulmonary veins were divided, permitting access to the dorsal and inferior wall of the left atrium. The right pulmonary veins were similarly mobilized and the interatrial groove developed (fig. 3). The pericardium and pleura were excised from the supradiaphragmatic portion of the inferior vena cava, and the sulcus terminalis between this structure and the inferior pulmonary vein was dissected. All remaining pericardium was excised. At the conclusion of the operation, bilateral anterior thoracotomy tubes were inserted and the wound closed with silk. Penicillin, 800,000 U., and streptomycin, 0.5 Gm., were given daily for two weeks.

Other Surgical Procedures

Bilateral Thoracic Sympathectomy

Five mongrel dogs weighing 10 to 14 Kg. were subjected to right thoracotomy under thiopental sodium anesthesia. The right stellate ganglion and the thoracic sympathetic chain and ganglia down to and including T5 were excised. Similarly, one week later the left stellate ganglion and thoracic chains and ganglia down to and including T5 were excised. The animals were maintained on antibiotics for two weeks and were studied after a minimum of three weeks had elapsed from the second operation in order that the data obtained would be comparable to that documented by Goodall and Kirshner.8

Sham Operations

Three animals which had previously undergone thoracotomy, pericardiotomy, and the cardiac manipulation incurred by dissection of the aortic root and atria were also studied as controls. This operation undoubtedly interrupted some sympathetic fibers to the heart, but did not remove the main cardiac plexuses in the mediastinum.

Results

Of 40 animals subjected to this operation, 15 survived. Eleven animals died at the time of operation because of technical errors which resulted in massive hemorrhage. The remaining 14 animals succumbed on the first or second postoperative day with massive pulmonary atelectasis and large accumulation of serosanguineous fluid in the pleural cavities. Survival rate was enhanced by improvement in postoperative care: Fluid was removed from the pleural spaces at frequent intervals by means of indwelling chest catheters, and tracheobronchial aspiration facilitated maintenance of satisfactory expansion of the lung. Survivors were free from any evidence of infection.

Verification of Denervation

Since the vagi and sympathetic trunks are not disturbed by the regional ablation described, stimulation of these structures affords a simple method for determining whether denervation has been accomplished. Accordingly, under light chloralose-urethane or thiopental sodium anesthesia, the right and
left vagi and stellate ganglia were exposed, at selected intervals after operation, decentralized, and stimulated with copper or platinum bipolar electrodes; at the same time heart rate, myocardial contractile force, and blood pressure were monitored. A Grass S-4 stimulator was employed, providing 1 to 30 unipolar stimuli/sec. of 1.2 to 10 volts and 0.3 to 10 msec. in duration. Responses in control dogs were elicited by stimulation of the vagi, stellate ganglia, or cardiac sympathetic nerves. The responses of the two latter structures give the same qualitative response.

The failure of vagal stimulation to produce bradycardia and hypotension (fig. 4) and the failure of stimulation of the stellate ganglia to produce immediate cardio-acceleration or augmentation of contractile force (fig. 5) confirmed the elimination of extrinsic nervous control of the heart. Complete denervation was indicated in this manner in 12 dogs. Three animals responded to sympathetic stimulation with a moderate increase in rate and contractile force, indicating incomplete denervation. Vagal stimulation of animals subjected to bilateral thoracic sympathectomy resulted in bradycardia and hypotension. Stimulation of the stellate ganglia was obviously not possible. Stimulation of the vagi and stellate ganglia of the dogs with sham procedures was followed by responses identical to those obtained in the controls.

**Chemical and Pharmacological Confirmation of Denervation**

Specimens of ventricular and atrial myocardium (0.5 to 2.0 Gm.) were excised at sacrifice of each animal and stored in the frozen state. Catecholamine levels in these tissues were determined fluorometrically by a modification of the trihydroxyindole method. In some specimens norepinephrine and epinephrine were determined separately by a fluorometric procedure based upon the differential oxidation of the two amines at pH 3.5 and pH 6.5.

Five animals were studied on either the first or second day following the procedure, and the rest at intervals of 3 to 68 days. It was found that the left ventricular catecholamine contents of control animals ranged from 0.57 to 0.98 µg./Gm. of muscle and averaged 0.71 µg./Gm. The amine content of
the ventricular muscle of denervated dogs studied on the first and second postoperative days ranged from 0.17 to 0.74 \( \mu \text{g} / \text{Gm.} \) and averaged 0.40 \( \mu \text{g} / \text{Gm.} \). The amine content of animals studied from 3 to 68 days postoperatively was less than 0.05 \( \mu \text{g} / \text{Gm.} \) in all instances except one in which a value of 0.08 \( \mu \text{g} / \text{Gm.} \) was obtained. A minimum of three days appears to be required for depletion of myocardial catecholamine following surgical denervation of the heart. The results of these studies are presented in table 1 with the data obtained from dogs subjected to bilateral thoracic sympathectomy and those which underwent sham procedures. Left ventricular catecholamine contents of dogs subjected to bilateral thoracic sympathectomy ranged from 0.41 to 0.62 and averaged 0.49 \( \mu \text{g} / \text{Gm.} \) of muscle. These values are significantly lower \( (P < 0.01) \) than those obtained from the control animals. The catecholamine content of the left ventricles of dogs subjected to the sham procedures revealed a fall to 0.33 \( \mu \text{g} / \text{Gm.} \) in the animal studied on the first postoperative day; the animal studied on the sixth postoperative day showed a value of 0.54 \( \mu \text{g} / \text{Gm.} \), while the one studied 28 days following operation showed values of 0.91 \( \mu \text{g} / \text{Gm.} \), which was not significantly different statistically from the mean of the control group.

The intravenous administration of tyramine in doses of 30 to 60 \( \mu \text{g} / \text{Kg.} \) to the seven dogs denervated more than three days prior to study elicited no significant cardiac acceleration or augmentation of contractile force, although the peripheral pressor action was still apparent (fig. 6). Following the intravenous administration of 60 \( \mu \text{g} / \text{Kg.} \) of tyramine to six normal anesthetized, vagotomized animals, myocardial contractile force increased 83 to 126 per cent and the increase averaged 103 per cent; mean arterial blood pressure increased from 25 to 45 mm. Hg and the increase averaged 38 mm. Hg. Heart rate increased from 32 to 56 beats/min., and the increment averaged 41 beats/min. In contrast.
Table 1

<table>
<thead>
<tr>
<th>Dog</th>
<th>Status</th>
<th>LV content</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>µg./Gm.</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.57 - 0.98</td>
<td></td>
</tr>
<tr>
<td>C1 -10</td>
<td>(range)</td>
<td>0.71 ± 0.05*</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS -1</td>
<td>44 days postop.</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>TS -2</td>
<td>48 days postop.</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>TS -3</td>
<td>26 days postop.</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>TS -4</td>
<td>23 days postop.</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>TS -5</td>
<td>41 days postop.</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>0.496 ± 0.04*</td>
<td></td>
</tr>
<tr>
<td>SH -1</td>
<td>1 day postop.</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>SH -2</td>
<td>6 days postop.</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>SH -3</td>
<td>28 days postop.</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>0.59 ± 0.17*</td>
<td></td>
</tr>
<tr>
<td>D§-1</td>
<td>Immediate postop.</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>D -18</td>
<td>10 hours postop.</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>D -22</td>
<td>1 day postop.</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>D -23</td>
<td>1 day postop.</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>D -29</td>
<td>2 days postop.</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>D -30</td>
<td>2 days postop.</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>D -17</td>
<td>45 days postop.</td>
<td>Less than 0.05</td>
<td></td>
</tr>
<tr>
<td>D -19</td>
<td>17 days postop.</td>
<td>Less than 0.05</td>
<td></td>
</tr>
<tr>
<td>D -20</td>
<td>7 days postop.</td>
<td>Less than 0.05</td>
<td></td>
</tr>
<tr>
<td>D -21</td>
<td>68 days postop.</td>
<td>Less than 0.05</td>
<td></td>
</tr>
<tr>
<td>D -24</td>
<td>3 days postop.</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>D -32</td>
<td>8 days postop.</td>
<td>Less than 0.05</td>
<td></td>
</tr>
<tr>
<td>D -33</td>
<td>6 days postop.</td>
<td>Less than 0.05</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>(Less than 0.05)</td>
<td></td>
</tr>
</tbody>
</table>

*Standard error of the mean.
†TS = dogs subjected to bilateral thoracic sympathectomy.
‡SH = dogs subjected to sham procedure.
§Accidental death by exsanguination from femoral arterial catheter. Denervation not verified by stimulation.

following the intravenous administration of the same dose of tyramine to the chronically denervated dogs, contractile force increased minimally (8 and 11 per cent) in two instances and not at all in the remaining five instances. Heart rate did not change; mean arterial blood pressure increased from 15 to 40 mm. Hg and averaged an increase of 28 mm. Hg. When tyramine was administered to denervated dogs in the first two postoperative days, to the sympathectomized dogs, and to the dogs with sham procedures, the responses in heart rate, contractile force, and blood pressure were indistinguishable from those of the control group.

Discussion

Although Cannon appreciated that complete interruption of neural pathways to the heart with preservation of the functions of other systems (digestion, respiration, peripheral vasculature) was contingent upon local excision, he indicated that denervation of the heart by means of regional dissection was practically impossible. Subsequent progress in surgical technique has made this undertaking experimentally practical. Current interest in heart transplantation has prompted this reappraisal of the problem.

In the technique described herein, complete denervation is accomplished with preservation of main vagal and sympathetic trunks. This permits verification by direct neural stimulation, a procedure not possible with other preparations. The virtually complete deple-
tion of myocardial catecholamines following this procedure, in contrast to the incomplete, transient depletion following the classic sympathetic ganglionectomy in the dog, indicates that this latter technique may be inadequate for the evaluation of the effects of pharmacological agents on denervated heart muscle, for the evaluation of physiological stresses such as exercise which may alter an amine-dependent biochemical reaction of the heart, and for anticipation of the performance of a cardiac transplant. Mild exercise does not elicit an increase in heart rate in dogs subjected to classic cardiac denervation, and the stroke volume does increase. This has been interpreted as demonstrating that cardiac muscle intrinsically performs in a manner consistent with Starling's law. This conclusion may be presumptive, since it is likely that these hearts retain large local stores of norepinephrine which may be called upon by a mechanical, chemical, or an accessory neural mechanism to modify cardiac performance. Physiological or pharmacological studies based upon classic denervation procedures are, therefore, subject to reappraisal.

The cardiovascular action of tyramine in the normal dog, i.e., cardio-acceleration, augmentation of contractile force, and elevation of the blood pressure, is said to be dependent upon the local release of norepinephrine. Complete chronic denervation of other structures such as the nictitating membrane and the iris of the eye abolishes their response to tyramine. The absence of cardiac response to tyramine in the chronically denervated dogs supports the contention that the drug operates through the release of norepinephrine. It also suggests that this agent may be used as a pharmacological test of chronic cardiac denervation.

Although animals studied in the early postoperative period may not be entirely normal in the metabolic sense, the sympathetic nervous system and neuroeffectors are reactive, as evidenced by responses of the dogs in which the sham operation was performed, and of the three animals which were incompletely denervated. Gazes and co-workers have demonstrated the reactivity of the hearts of dogs to sympathomimetic amines one to eight days following thoracotomy, pericardiotomy, and suture of a strain-gauge arch onto the myocardium. It is unlikely that the stimulation procedures alone depleted stores of the transmitter substance, since it has been shown that the stellate ganglia may be stimulated for as long as 11 hours with sustained effect. Furthermore, the animals with totally denervated hearts herein reported had no response at any time during the study period. Therefore, the low levels of myocardial catecholamines cannot be attributed to depletion by exhausting the response during stimulation. Early study of these animals was necessary in order to determine the time course of the depletion of myocardial catecholamines. The catecholamine in the hearts of the animals subjected to bilateral thoracic sympatheticectomy probably does not represent storage of circulating endogenous amine, since the dogs with total extrinsic cardiac denervation show no such effect. The source is presently unknown and requires further investigation.

The major obstacle to homotransplantation of the heart, as for other tissues, is immunological. Certain technical and physiological problems are, however, peculiar to homografts of the heart. Among the factors influencing the survival of the heart and its ability to support the circulation is the fact that the transplanted heart has been deprived of its intrinsic neural control. Accordingly, a knowledge of the responses of the denervated heart to physiological demands, abnormal stresses, and pharmacological agents is critical to the intelligent management of such a transplant. The animals prepared by regional neural dissection do not exhibit the gastrointestinal complications and weight loss which frequently prohibit survival or maintenance of condition satisfactory for study following sympathetic ganglionectomy and cervical vagotomy. Insofar as animals denervated by regional neural dissection are otherwise essentially intact, their cardiovascular responses are analogous to those which one may expect in the transplanted heart.

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CARDIAC DENERVATION

Summary
A technique for complete extrinsic denervation of the heart by the ablation of neural structures in the mediastinum is described. The completeness of denervation was verified by direct electrical stimulation of the main vagal and sympathetic trunks. Total depletion of myocardial catecholamines followed the procedure in chronic survivors. Animals prepared in this manner are useful in the study of the neurologically isolated heart and simulate this aspect of the problem of cardiac transplantation.

References
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