WALTER B. CANNON formulated a "law of denervation" based upon extensive experimental work with neurotransmitter systems. Simply put, this "law" stated that a denervated organ becomes supersensitive, after a time, to its usual neurotransmitter substance. The most exquisite example of this denervation supersensitivity is seen in skeletal muscle whose sensitivity to acetylcholine increases as much as 1000-fold following denervation. Other structures show varying degrees of supersensitivity, depending on many factors, among which is whether the denervation is pre- or postganglionic.

The heart, once thought to be an exception to this law, demonstrates an increase in sensitivity to norepinephrine (NE) of 1.5-10-fold following denervation, depending on whether one is measuring atrial contractility, ventricular contractility, or heart rate. The question of whether or not the heart becomes supersensitive to cholinergic agents following denervation is, however, unsettled. The scanty data which exist are often inconclusive and contradictory.

The following experiments were performed to determine whether the extrinsically denervated heart becomes supersensitive to cholinergic agents which may interact with myocardium and cardiac parasympathetic neural elements. Two types of agents were employed: one that primarily interacts with the effector cell (acetylcholine) and one that primarily interacts with the intrinsic parasympathetic ganglion cell (nicotinic). In this manner, it was hoped that denervation-induced sensitivity changes in either the effector cell or the intrinsic innervation, or both, could be determined.

**Methods**

**CHRONIC EXPERIMENTS**

Fifteen mongrel dogs were anesthetized with thiopental sodium (20-25 mg/kg) and subjected to total extrinsic denervation of the heart using the method of Geis et al. The procedure consists of two operations separated by an interval of 1-2 weeks. In the first operation, the left atrium is separated from its mediastinal attachments from the superior to the inferior junctions with the interatrial septum and reanastomosed. At the same time, the aorta and pulmonary artery are circumferentially stripped of their adventitial layers. In the second operation, the entire wall of the right atrium, as well as the interatrial septum, is incised and reanastomosed. The latter incision is done under inflow occlusion. This technique produces a heart that has been effectively separated from all mediastinal connections which may act as pathways for both sympathetic and parasympathetic cardiac nerves. It produces a heart that is postganglionically denervated with regard to its sympathetic nerves, but preganglionically denervated with regard to its parasympathetic nerves.

A period of 3-6 weeks was allowed for recovery before these dogs were employed in the acute phase of the experiments. This is ample time for development of supersensi-
Acute experiments

In addition to the denervated animals, 15 control dogs were used in these experiments. All dogs were anesthetized with thiopental sodium (25-30 mg/kg, supplemented as necessary) and the chests were opened by transternal thoracotomy in the fourth or fifth interspace. The cervical vagi and stellate ganglia were isolated and prepared for stimulation and the left carotid artery was cannulated for pressure recording and subsequent drug injections. The tip of the carotid cannula was positioned just at the aortic side of the aortic valve. Once cardiac bypass was established, drugs injected into this cannula would then enter the coronary circulation directly because of the retrograde nature of arterial perfusion during bypass. Total cardiopulmonary bypass was accomplished in the usual manner. Venous return from the venae cavae was drained into a disc oxygenator, oxygenated in an atmosphere of 100% O₂, warmed, filtered, and returned to the dog via a femoral artery. Sumps in both ventricles were placed under air-relieved suction and the coronary and bronchial blood was collected and returned to the oxygenator.

Arterial pH was measured at intervals throughout the experiments with an Instrumentation Laboratories blood-gas analyzer and ranged between 7.35 and 7.40. Arterial blood temperature was monitored with a thermistor probe (Yellow Springs Instruments) placed directly in the arterial inflow line. The blood temperature was maintained between 34 and 36°C by warming the oxygenator with heat lamps. Arterial pressure was monitored by placing a catheter in the ascending aorta with a pressure transducer described in earlier publications.10 Saline-filled balloons mm Hg in the atria and 3-8 mm Hg in the ventricles, between 75 and 125 mm Hg. (Trantec). In these experiments, arterial pressure varied between 75 and 125 mm Hg.

Results

Responses to ACh

Typical responses of a normal and a denervated dog to ACh, 0.5 μg, are shown in Figure 1. Both hearts were pressures equivalent to those recorded in the open-chest dog preparation. The heart rate was held constant by pacing from the right atrium. With heart rate, preload, and afterload eliminated as variables, changes in isovolumic pressures very closely reflect changes in chamber contractility.

All drugs were administered intracoronary via the carotid cannula in volumes of 0.5–1.0 ml followed by a 1–2-ml wash with normal saline. Acetylcholine (ACh), in doses of 0.005–1.0 μg, was used to produce the expected negative inotropic responses of the cardiac muscle. Nicotine HCl (NIC) was used to activate the intrinsic parasympathetic neural elements (IPN). The doses employed were 10–300 μg. These doses have been shown to produce a highly selective activation of IPN which results in negative inotropic responses of both atria and ventricles.11 These responses to NIC can be blocked totally by neural or ganglionic blocking agents, while the responses to ACh are completely unaffected.11 Thus, the two agents can be employed to study the effects of denervation on both the decentralized effector cell and the denervated IPN. In dogs with intact cardiac nerves, NIC also induces the release of NE from postganglionic sympathetic neurons. This results in an indirect, positive inotropic drug effect. In seven experiments on control dogs, these effects were abolished with 2–4 mg of intracoronary practolol, a β-adrenergic blocking agent. The responses of these control animals after practolol were used as the control group to detect changes in sensitivity of the intrinsic innervation to NIC in the denervated dogs.

Changes in isovolumic pulse pressures in response to the injections were measured and expressed as percent change from control ± SEM. Significance of differences between the means of responses in the two groups of dogs was analyzed by Student's t-test. A level of P < 0.05 was considered to indicate a significant difference.

FIGURE 1 Recordings which illustrate typical responses of an intact heart (left panel) and a denervated heart (right panel) to 0.5 μg acetylcholine (ACh) (arrows). Isovolumic pressures from the right atrium (RA), right ventricle (RV), left atrium (LA), and left ventricle (LV) are shown. Calibrations (center) are in mm Hg.
paced at a constant rate from the right atrium (RA). In the normal heart, both RA and left atrial (LA) isovolumic pressures decrease within a few beats following injection and show significant recovery even before the maximum ventricular depressions have been reached. Both the right (RV) and left (LV) ventricles display negative inotropic responses to ACh, but they develop more gradually (15-20 sec) and recover more slowly. These differential atrial and ventricular responses to injected ACh have been reported earlier and also are typical of atrial and ventricular responses to vagus nerve stimulation. The responses of the denervated heart to the same dose of ACh (right panel) are not remarkably different either quantitatively or qualitatively from those of the control animal. In this particular dog, the LA was not responsive to ACh at this dose level. It is important to note that in no case is there a significant positive component to the cardiac responses to ACh. These recordings are quite representative of what was observed overall; i.e., injection of ACh in doses of up to 1.0 μg, intracoronary, rarely produced any significant positive inotropic response. These observations are in sharp contrast to those for NIC which are described below.

The apparent lack of supersensitivity to ACh observed in the denervated dogs is substantiated by the overall comparative data shown in Figure 2. In only one chamber and at only one dose level (RA, 0.01 μg) is the response of the denervated heart significantly greater than that of the normal heart. In fact, the opposite appears to be true, i.e., in most instances, the denervated heart exhibits less depression in response to ACh than does the intact heart. In four cases, these lesser responses are significantly different at the P < 0.05 level. As previously reported for denervated animals, the most responsive chambers to ACh in the normal dogs were the RA and LA. The ventricles generally displayed about 50% of the negative inotropic responses of the atria. Except for the RA, these differences are not nearly as clear in the denervated dogs. In both groups of dogs, inotropic responses do not show a clear relationship to ACh dose. Based on the data shown in Figure 2, it does not appear that the denervated heart shows any supersensitivity to the negative inotropic effects of ACh.

**RESPONSES TO NIC**

Intracoronary injection of NIC has been shown to activate the intrinsic parasympathetic ganglion cells (IPN) in the heart. Although the cardiac muscle cells undergo parasympathetic decentralization following chronic extrinsic cardiac denervation, these intrinsic neural elements are denervated by the procedure. The comparative responses to NIC of the two groups should, therefore, reflect any supersensitivity of the IPN.

Typical responses of control hearts (before and after β-blockade), as well as denervated hearts, to 50 μg of NIC are shown in Figure 3. Prior to β-blockade (left panel), the negative inotropic responses produced by nicotinic activation of the IPN are masked by concurrent release of NE from postganglionic sympathetic nerves. This results from stimulation of nicotinic receptors on the adrenergic nerve fiber. This indirect NIC action is apparent in the responses of the RV in Figure 3. After elimination of this indirect action by β-blockade (central panel) or denervation (right panel), only negative inotropic responses are produced. These negative inotropic effects of NIC are qualitatively similar to those produced by ACh (Fig. 1), in that the atrial depression has a short latency and a fast recovery (RA), while the ventricular responses are slower in both developing and recovering (RV and LV, right panel).

**Figure 2.** Summary of responses of intact (open bars) and denervated hearts (closed bars) to acetylcholine (ACh). Each bar is the mean of 15 experimental values. SEM is shown by the brackets. Chambers are as in Figure 1. Significant differences (P < 0.05) of denervated from control responses at a given dose are indicated by •.
The changes in sensitivity of the heart to nicotine (NIC) in denervated dogs are compared to intact dogs. The sensitivity changes are observed in the LA, RV, and LV chambers, with a significant shift to the left compared to intact dogs. The increase in sensitivity of 3- to 5-fold, depending upon the dose, is observed in the denervated dogs. The net response of a chamber to NIC is the result of the indirectly mediated positive response and the indirectly mediated IPN negative response. The denervated dogs exhibit an increased sensitivity to NIC throughout the dose range studied. The direct positive response is retained, whereas the indirect positive response and the indirectly mediated IPN negative response are diminished.

When overall responses of the control (without β-blockade) and denervated hearts are compared, some clear differences emerge. After β-blockade, the control heart shows a slight negative inotropic response to 50 μg NIC, whereas the denervated heart exhibits significant RA, RV, and LV negative inotropy in response to the same dose. When the overall responses of the control and denervated hearts are compared, some clear differences emerge (Fig. 4). At the lower doses of NIC (25 and 50 μg), the control animals show responses that are entirely negative. At the higher doses (100 and 200 μg), most of the responses are positive. Obviously, the net response of a chamber to NIC is the result of the indirectly mediated positive response and the indirectly mediated IPN negative response. In the case of the denervated dogs, the degeneration of the postganglionic sympathetic nerves eliminates the source of NE for the indirect positive response. Therefore, all NIC responses observed in the denervated dogs were negative. On this basis, many of these responses were significantly different from those of the normal dogs. Positive responses to NIC in the 15 denervated animals were very rare; i.e., only nine of 168 observations (5%) were positive. In the 15 intact dogs, 56 of 131 responses to NIC were positive (43%).

To provide control responses with which the responses of the denervated animals might be compared, seven intact dogs were given NIC following β-blockade with practolol. Figure 5A-D compares the NIC dose-response curves for the four heart chambers in these two groups. After β-blockade, only 10 of 153 responses of the intact dogs to NIC were positive (6.5%). Overall, the denervated dogs displayed an increased sensitivity to NIC. Although the differences between the mean values of the two groups were not all statistically significant, the dose-response curves for the denervated group were consistently shifted to the left, compared with intact dogs, after β-blockade. In Figure 5A, the RA curve for the denervated dogs is significantly shifted to the left, indicating an increase in sensitivity of 3- to 5-fold, depending upon which portion of the curves one selects for comparison. Responses of the denervated animals at 25 and 50 μg NIC are significantly greater than control. There is no significant difference between maximum responses in the two groups except that they occur at different dose levels. The sensitivity changes in the LA (Fig. 5B) are not as clear. Although the dose-response curve for the denervated dogs is shifted to the left, only the 25 μg point is clearly statistically different from control. The magnitude of the shift is approximately 2-fold. There is also evidence in the RV responses that denervation increases sensitivity to NIC (Fig. 5C). Although responses to the two lower doses of NIC do not appear to be different between the two groups, the denervated dogs show a significantly higher response at 100 μg. The RV is the only chamber in which denervation did not produce an apparent decrease in the threshold dose of NIC. This latter change is clear in the dose-response curves of Figure 5D. The LV of the denervated dogs exhibits an increased sensitivity to NIC throughout the dose range studied. The difference at only one dose level (100 μg) is statistically significant. Depending on the method of comparison used, the LV of the denervated dogs appears to be 2-4 times more sensitive to NIC than that of the control animals. If one uses the horizontal displacement of the dose-response curve, the increase could be as much as 6-fold.

As reported earlier, these responses to NIC could subsequently be blocked by 10-30 μg of tetrodotoxin, intracoronary, which had no effect on responses to ACh.

**Discussion**

On the basis of the data presented, it is clear that the extrinsically denervated heart does not become supersensitive to ACh (Fig. 2). The lack of supersensitivity is undoubtedly related to the fact that the surgical denervation produces a decentralization rather than a denervation of the atrial and ventricular effector cells. This contrasts with data on other organ systems which indicate a modest (2- to 4-fold) increase in sensitivity following denervation.
centralization. As early as 1936, Cannon and Rosenblueth showed that the denervated superior cervical ganglion displayed a response threshold to ACh that was only one-fourth that of the innervated ganglion. The decentralized nictitating membrane was used as the test system. The phenomenon of supersensitivity following decentralization has also been demonstrated in vas deferens and other effector cells. Very little information has been available regarding cholinergic sensitivity of the denervated heart. Recently, Jacobs and his co-workers have reported that extrinsic cardiac denervation does not appear to alter the bradycardia in response to ACh. However, these workers did show an increased tendency for atrioventricular (AV) block in response to ACh, suggesting a possible increase in AV nodal sensitivity. More recently, Hageman et al. have shown that the perfused sinoatrial (SA) node of selectively parasympathectomized animals does not show an increased sensitivity to ACh injected directly into the isolated SA nodal artery. It appears that the inotropic sensitivity of the denervated heart to ACh also is an exception to the general observations following decentralization. If atrial and ventricular muscle do show any moderately increased cholinergic sensitivity following extrinsic denervation, perhaps the techniques used in these experiments were not sensitive enough to demonstrate it. There is considerable response variability in the methods used because of the stress placed upon the animal, variation in the pattern of drug delivery through the coronary circulation, and the nature of the isovolumic pressure-recording technique. It may be that this variability would be enough to obscure small changes in sensitivity. However, the data of Figure 2 are quite clear and appear to rule out such marginally detectable changes. It is more likely that the heart is indeed an exception to the usual enhancement produced by chronic decentralization.

As we have shown previously, the negative inotropic
effects of NIC at these doses are primarily the result of activating the IPN. Denervation of the IPN (which would be expected from the surgical technique used) does result in an increased sensitivity to nicotinic stimulation (Fig. 5A-D). Although these increases are not statistically significant in all chambers at all dose levels, the NIC dose-response curves in the denervated dogs are consistently shifted to the left of the control curves. There are enough statistically significant differences to conclude that denervation supersensitivity of the IPN does occur. This supersensitivity is only apparent, however, when the indirect positive inotropic effects of NIC in the control dogs are blocked by practolol. In the absence of practolol (Fig. 4), the effects of NIC on the IPN are obscured by release of catecholamine from sympathetic postganglionic nerves. This phenomenon has been described previously by a number of workers.15-17 That the sources of this positive inotropic effect of NIC are the sympathetic cardiac neural elements is verified by the complete absence of positive inotropic effects in the denervated animals (Fig. 4).

The increased sensitivity of the IPN is probably the result of a postsynaptic change which involves a "spreading" or increased number of drug-sensitive receptors on the membrane of the ganglion cell similar to the well known effect of denervation on the cell membranes of skeletal muscle.21 Kuffler and his co-workers22 have shown that the area of chemosensitivity of IPN cells in the interatrial septum of the frog heart increases greatly after section of the vagosympathetic trunks. The time course of development of this IPN supersensitivity (4-8 days) correlates well with the usual time course for supersensitivity development.15 Therefore, the increased responsiveness to NIC seen in the cardiac-denervated animals probably represents a true postjunctional or type II supersensitivity of the intrinsic nerves of the heart.

No such supersensitivity was observed with ACh (Fig. 2). Neither was ACh effective in producing indirect positive inotropic responses similar to those of NIC. There are at least two reasons for these findings. First of all, nicotinic receptors of the cardiac nerves are probably much less sensitive to ACh than are muscarinic receptors on the cardiac muscle cells. Blumenthal et al.23 have shown that intracoronary doses of ACh from 0.01 to 1.0 μg produce only negative inotropic effects on the canine LV that can be blocked by atropine. In order to produce positive inotropic effects, the dose of ACh had to be raised to 20-300 μg, and prior administration of neostigmine and atropine was necessary. These positive responses could then be blocked completely by intracoronary administration of tetraethylammonium chloride.23 These observations indicate a vastly greater ACh sensitivity of cardiac muscarinic receptors, compared with nicotinic receptors. This would explain why ACh did not produce any positive inotropic responses at the doses employed in this study (0.01-0.5 μg). Even with an increased nicotinic sensitivity of the IPN following denervation, the doses of ACh used probably were still insufficient to produce IPN activation. These speculations assume, of course, that the nicotinic receptors on the IPN and those on the sympathetic nerve endings have approximately the same characteristics. Second, the inotropic responses to both direct and indirect actions of ACh (muscarinic and nicotinic) are qualitatively identical. Therefore, any effects of IPN stimulation which would be produced at the doses used would probably be much less than the direct effects of ACh on the effector cells. Thus, IPN stimulation by ACh would not be detectable under the present experimental circumstances even if it should occur at the doses used.

The adrenergic supersensitivity produced by cardiac denervation* would be considered a type I or "deviation" supersensitivity according to Fleming's classification;4,6 i.e., a change in the percent of an administered amount of a drug that is available in the biophase to interact with its receptors. This results from the loss of the catecholamine reuptake mechanism that had existed in the degenerated sympathetic nerve endings. In contrast, the decentralization supersensitivity observed in the present experiments would fit Fleming's classification as type II or "nondeviation" supersensitivity; i.e., enhanced responsiveness of the target cells (IPN). It is interesting to note that these two diverse mechanisms produce the same order of magnitude of supersensitivity, 2- to 10-fold.

The chamber that illustrates the enhanced sensitivity to NIC most clearly is the RA (Fig. 5A). This correlates well with anatomical evidence that indicates the largest concentration of parasympathetic ganglion cells is in the RA.24-26

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Heterogeneity of the Hypoxic State in Perfused Rat Heart

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SUMMARY Tissue oxygen gradients were examined in the saline-perfused rat heart by NADH fluorescence photography. In high flow hypoxia, where the coronary flow was maintained and the arterial oxygen tension was gradually reduced, oxygen extraction was virtually complete before oxygen consumption was significantly diminished. Inadequate oxygen delivery resulted in a well defined pattern of anoxic zones. The anoxic zones were several hundred microns in width, an order of magnitude greater than intercapillary distances. Anoxic zones appeared despite only a slight change in effluent oxygen tension from the control. In high flow hypoxia, ischemia, and acidosis-induced ischemia, the anoxic zones disappeared when control perfusion conditions were restored. The data demonstrate that tissue oxygen gradients are very steep in the hypoxic state, so that ischemia and hypoxia result in discrete heterogeneous areas of anoxic tissue bounded by sharp areas where the oxygen supply is sufficient to maintain normal mitochondrial oxidative function. In these states in which oxygen delivery is less than oxygen demand, coronary perfusion appears to be regulated at the level of the arterioles rather than the capillaries.
Cholinergic sensitivity of the denervated cannine heart.
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Circ Res. 1977;41:600-606
doi: 10.1161/01.RES.41.5.600

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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