Cellular Abnormalities in Chronically Denervated Myocardium
Implications for the Transplanted Heart

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A major problem in the study of the transplanted heart is the difficulty in distinguishing between the effects of chronic denervation, rejection, and immunosuppressive treatment. The effect of chronic denervation per se cannot be studied in isolation in humans, but in animals this can readily be achieved. Our opinions are determined by this consideration, so that in this article we focus on such studies.

Understanding of the function of the transplanted heart is becoming increasingly important as clinical success with the procedure grows. It is already clear that the application of knowledge of "standard" myocardial metabolism to the chronically denervated heart may not be appropriate in this context.

The Chronically Denervated Dog Heart

Over the years, much information has accumulated from experiments on dogs with chronically denervated hearts. In our laboratories, denervation was carried out surgically by the method of Donald and Shepherd, which we have modified by using a left thoracotomy followed by right thoracotomy. This change in order facilitated the dissection behind the pulmonary veins on the right side, which is the most hazardous part of the procedure, and greatly increased our success rate. By successively removing components of the autonomic supply to the heart in stages, Randall's group have elegantly dissected the separate parts of the neuronal control systems and have elucidated the effects with selective blockade. In this article, we concern ourselves only with global denervation.

The confirmation of denervation and the global behavior of the chronically denervated heart has been described in a review by Donald and in publications from our group. Essentially, the function is normal except for a blunted frequency response to changes in exercise level or other reflex demands on cardiac responsiveness (blunting of frequency response to change in exercise level has also been confirmed in human cardiac transplant patients, but unlike the denervated dog, total exercise capacity is limited in such patients [for references, see Banner et al]). Supersensitivity to the neurotransmitter noradrenaline develops in 3 weeks after denervation and persists for some time. The mechanism of this supersensitivity to noradrenaline is probably lack of noradrenaline reuptake.

Characterization of the Chronically Denervated Heart

To examine the various aspects of chronic denervation, we will begin by considering the obvious question, "If all the extrinsic nerves have degenerated, should one now be able to see what nerves remain?"

Attempts to Demonstrate Intrinsic Nerves

We postulated at an early stage that the heart might have an intrinsic nervous system with neuronal cell bodies within the heart that have their own neurotransmitters. However, the test of this demanded difficult histochemical techniques that were initially unavailable to us. Collaborative work has meant that histochemical techniques now have been used in our research and reported by Forssmann et al to the New York Academy of Sciences. Staining was carried out for vasoactive intestinal polypeptide (VIP), peptide-histidine-isoleucine (PHI), and neuropeptide Y (NPY). Immunoreactively staining VIP and NPY nerve fibers were unequivocally identified in denervated hearts, but the former are much more numerous in innervated hearts, particularly VIP-staining varicosities. Retention of NPY after denervation has been confirmed by tissue assay (J.M. Allen, unpublished observations). It is likely that much more information on intrinsic nerves will emerge with further studies.

Another aspect of the problem is to consider the remaining neuroendocrine mediators.
Endocrine Functions of the Heart

Dopamine. Noradrenaline tissue content is depleted with denervation, and the indications are that the reduction of noradrenaline is long lasting (12 years) in autotransplanted dogs. The depletion of noradrenaline after chronic denervation was used to confirm degeneration of the sympathetic postganglionic neuron, and we expected that this would be accompanied by dopamine depletion, thinking that this was just a precursor in neuronal noradrenaline synthesis. However, our finding was that dopamine does not deplete, which has been confirmed by Mohanty et al’s study. Moreover, the chronically denervated myocardium has high levels of tyrosine hydroxylase (i.e., dopamine synthetic capacity). This finding stimulated Ilebeck et al to measure the arterial to coronary venous blood dopamine concentration difference; they found in innervated pig hearts that the venous concentration of dopamine was always higher than the arterial concentration. The physiologic role of myocardially secreted dopamine could be fascinating; so, too, would be the influence of a lack of noradrenaline in hypertension and transplantation.

Atrial natriuretic factor. Left atrial pressure was raised by constriction of the mitral valve ring. Cardiac denervation abolished the atrial natriuresis, proving that this response involves cardiac afferent nerves and is not mediated by a hormone, as already shown by Henry and Pearce. At the time, atrial natriuretic peptide (ANP) assays were not available. Later, when many groups could assay ANP, our results were ignored. Then, Goetz et al repeated our experiment and showed that in the cardiac denervated condition, when atrial natriuresis is absent, ANP secretion was normal. Furthermore, plenty of ANP remains in the denervated tissue. We have been involved in further studies using rapid atrial pacing of the heart and acute volume loading. The intervention of rapid pacing is interesting because it causes ANP to pour out of the heart. We can deplete ANP by this method in innervated hearts, so it should be possible to see if cardiac denervated hearts are more easily depleted. Rapid atrial pacing produces a water diuresis, not a natriuresis; there are many circumstances of such lack of correlation between ANP and natriuresis (e.g., acute volume loading). It could be postulated that denervation of the heart must have some effect on the response of the kidney to ANP. If so, it still means that atrial natriuresis is dependent mainly on some aspect of cardiac function other than ANP. If ANP does not always produce natriuresis on its own without other potentiating factors, the implication is that it is not a dominant natriuretic substance. The application of this work to clinical human transplantation is not straightforward because some recipient atrium is left in situ as a site of afferent neurons.

Other aspects of the function of the chronically denervated heart have more immediate applicabilities to transplantation, such as the question, "How efficient is a denervated heart?"

Myocardial Oxygen Consumption and Coronary Blood Flow

Removal by denervation of a predominant α-sympathetic vasoconstrictor tone would be expected to result in higher coronary blood flow for a given arterial pressure after denervation. This expectation is contradicted by the results of a study of Gregg et al who appeared to show lower coronary blood flow after denervation. However, we were not entirely convinced by their methods, which used cuff-type electromagnetic flow transducers on the coronary arteries in an unpaired design in only three animals. We, therefore, implanted left atrial catheters in dogs and measured myocardial blood flow by the microsphere technique. The values for flow per gram of left ventricular myocardium were higher than for innervated controls in an unpaired study. We now think that this could be either due to relief of sympathetic tone or possibly secondary to a change in myocardial oxygen consumption. In a more controlled study, we made paired observations (i.e., measurements before and after denervation in the same animals) and accompanied them by determinations of arterial and coronary sinus oxygen concentrations so that myocardial oxygen consumption could be determined by the Fick equation. Because the denervated heart has a much lower spontaneous heart rate, under the chloralose anesthetic we used, the heart rate was made the same in the two measurements by atrial pacing. The results showed that both myocardial oxygen consumption and coronary blood flow were higher after denervation for the same level of heart rate and minute work. We would expect from the work of Mohrman and Feigl that a reduction in α-adrenergic sympathetic vasoconstrictor tone, when combined with a change in metabolic rate, would show a narrowing of the arterial to coronary sinus oxygen content difference; this did not occur.

Therefore, our opinion is that our results provide no evidence to support the idea that the coronary vasculature is under tonic neural vasoconstrictor control. However, a contrary conclusion is obtained from acute studies on the innervated heart, in which circumstance an increase in coronary blood flow may be found following the administration of α-adrenergic--blocking agents. These differences in results may merely reflect the level of sympathetic activation at the time of removal of the neuronal influence; alternatively, adrenergic-blocking drugs may be removing an ω-receptor-mediated humoral effect of circulating catecholamines. Surgical denervation removes only the neuronal influence and not the humoral effect. The supersensitivity of the chronically denervated heart to circulating noradrenaline renders the interpretation of adrenergic-
blocking–drug studies in such a preparation difficult to make in terms of normal physiology.

We had expected a decrease rather than an increase in myocardial oxygen consumption because we thought that the presence of noradrenaline in the innervated tissue would tend to keep the metabolic rate up rather than down. We were aware that normalization for minute work was not necessarily adequate for normalization of metabolic rate because if the heart were larger in the denervated state, the same minute work and left ventricular pressure would be associated with a higher wall stress and/or wall shortening and, therefore, a higher oxygen consumption.\(^32,33\) This is, in itself, an oversimplification of the problem because myocardial oxygen consumption is also related to the pressure-volume area\(^34\) and to contractility or the velocity of contraction.\(^35\) In the intact dogs, we tried to tackle this problem in two ways. In the first, we used pericardiectomy dogs as controls, allowing expansion of the heart beyond the pericardial constraint, as for the denervated hearts; the myocardial oxygen consumption and coronary blood flow were not enhanced by this procedure when measured at comparable heart rates. The second was to implant ultrasonic crystals across the left ventricular equator to measure diameter; in the few successful experiments, subsequent denervation did not increase diameter. Despite these apparently reassuring results, no group, to our knowledge, has been able to carry out the controlled experiments, in dogs with denervated hearts, that have been shown to be necessary in control animals\(^33-35\) to ensure that changes in mechanical performance are not responsible for the changes in myocardial oxygen consumption. In our view, such experiments are likely to be difficult; we, therefore, sought a method of investigation of this problem that did not involve the complexities associated with study of the intact animal.

We, therefore, studied isolated denervated cardiac tissue and mitochondria isolated from denervated hearts and found that they also had an increased myocardial oxygen consumption in vitro. In the definitive study,\(^36\) we used paired biopsies before and after denervation in the same dogs. Oxygen consumption was measured in vitro with complete uncoupling to measure the fastest intrinsic metabolic rate of the mitochondria; chronic denervation caused an increase in oxygen consumption in every case. Thus, the tonic release and uptake of noradrenaline in the innervated state appears to be increasing metabolic efficiency. Catecholamines given acutely have been believed by some to have an opposite effect, namely, oxygen wasting. This is disputed by Feigl's group\(^37\) who found that all the effects of catecholamine on myocardial oxygen consumption could be accounted for on the basis of the mechanical determinants of metabolic rate, a point that is difficult to be certain of if the pressure-volume area must be taken into account.\(^34\)

Another possible way of explaining the increased myocardial oxygen consumption is to postulate an enhanced response to thyroid hormones. However, thyroid hormone receptor studies in which the nuclear T3- and cytosolic T4-receptor–binding characteristics and the rate of conversion of T4 to T3 and T4 to reverse T3 were measured failed to reveal any difference from control.\(^38\)

If the denervated heart is metabolically inefficient, one might well ask, “Is this because of a change in the responsible enzyme systems?”

**Myocardial Enzymes**

We have assayed many myocardial enzymes in an attempt to explain the increased metabolic rate of chronically denervated hearts. Enzyme assays have the disadvantage that they are measured in vitro in optimal conditions of pH, substrate concentration, cofactors, and so on. Therefore, they only tell us whether the amount of enzyme protein present is normal, not how active the enzyme was in the condition prevailing in vivo.

With this proviso, we can say that the activity of the following enzyme activities are normal in chronically denervated myocardium—succinate dehydrogenase, cytochrome oxidase, monoamine oxidase, calcium-dependent ATPase,\(^39\) glycerol-de-3-dehydrogenase, and phosphofructokinase.\(^40\) Our proviso will be seen to be justified rather dramatically in the case of phosphofructokinase, as we discuss later.

Another way of obtaining information about myocardial metabolism is to measure the levels of intermediate metabolites. This is more hazardous because they can change from the time of biopsy to the time of freezing the biopsy (which can take several seconds). This is a problem that could be avoided in the future if our preparation were to fire the imagination of the nuclear magnetic resonance experts. With these reservations, it can be stated that the following metabolites were normal—ATP, creatine phosphate, and glucose-6-phosphate.\(^40\)

Another obvious question that leads from the finding of metabolic inefficiency in the denervated heart is the question, “Are substrates oxidized abnormally?” We have partial evidence that lactate is normally oxidized but are unaware of studies on lipid oxidation in the chronically denervated heart; this leaves glucose.

**Glucose Oxidation**

The one intermediate metabolite that was altered after chronic denervation was fructose-6-phosphate, which was increased twofold to threefold. This in the presence of normal glucose-6-phosphate suggests inhibition of conversion by phosphofructokinase, even though that enzyme is present in normal amounts; thus, its in vivo activity might be inhibited by cofactor imbalance. To test this idea, we wanted to measure the oxidation of \(^14\)C-glucose to \(^14\)CO\(_2\). However, we encountered a difficulty in that glu-
cose oxidation is very dependent on the concentration of other substrates. The heart of the normal intact animal always takes up lactate and oxidizes it. A systematic investigation in innervated hearts of intact dogs revealed that lactate was not only always taken up and oxidized but that it was preferred as substrate over glucose and free fatty acids when the latter two were presented to the heart simultaneously in supramaximal concentration. Therefore, lactate oxidation increases with arterial lactate concentration until the system saturates. As a consequence, glucose oxidation declines with increasing arterial lactate concentration as the heart switches from glucose to lactate. We were, therefore, careful to check that spurious changes in glucose oxidation did not occur as the result of changes in arterial lactate concentration.

A second problem encountered was the method of expressing substrate uptake and oxidation rates. Because the metabolic rate of the denervated hearts was increased, we normalized glucose oxidation rate (14C-glucose to 14CO2) by the myocardial carbon dioxide production; this gives by calculation the percentage of total substrate oxidized that is glucose. This method also has the advantage that although coronary blood flow is required for the measurement of both glucose oxidation rate and myocardial CO2 production, it cancels out when the one is divided by the other and, therefore, does not need to be measured. Thus, all we needed to do during the 14C-glucose infusion was to measure the arteriovenous 14CO2 and total CO2 differences and to correct for specific activity and the number of carbons (6) in the glucose molecule. When this was done, we found that the glucose oxidation was always lower in the same dog after denervation (paired experiments) even though, if anything, the arterial lactate concentrations were slightly lower. There is, therefore, a true inhibition of glucose oxidation in the chronically denervated heart. The rise in fructose-6-phosphate suggests that this occurs at the phosphofructokinase step. Inhibition of the anaerobic pathway might cause increased dependence on aerobic production and some increase in oxygen consumption. However, because the anaerobic pathway is a minor contributor to energy turnover in the normal aerobic heart, we do not think that this finding explains the overall increase in oxygen consumption.

Other oxygen-consuming processes that might conceivably be partaking in the overall metabolic inefficiency of the denervated heart are specific ATPases. Studies of this type have not been comprehensive, to our knowledge, except in the case of the sodium pump. Studies of calcium ATPases would seem to us to be warranted.

**Sodium-Potassium ATPase**

The finding of an inhibited pathway in vitro with normal enzyme activity alerted us to the necessity of measuring enzyme function rather than concentration. For the Na-K-ATPase we used quiescent myocardial biopsies and measured the ouabain-dependent radioactive rubidium uptake. The literature on the effect of noradrenaline on this system is ambiguous, but in our hands there is no doubt that the exchange is stimulated. This phenomenon does not cause an oxygen-wasting effect because the contribution of energy consumption for sodium pumping is a negligible proportion of the total. There were many aspects of this system that we were obliged to investigate before applying the method to denervated hearts (e.g., the extracellular potassium dependence). However, with experience, we were able to determine that chronic denervation caused an increase (average, 147%) of ouabain-sensitive rubidium uptake. The number of “pump sites” was measured by tritiated ouabain binding and found to have increased considerably less (27.6%). Therefore, presumably, the activity of individual Na-K exchangers is enhanced. Noradrenaline had an even more marked stimulatory effect on ouabain-dependent rubidium exchange after denervation than it had in the control myocardium, indicating supersensitivity.

These results parallel those for total energy turnover that is stimulated acutely by noradrenaline but, paradoxically, stimulated also by chronic lack of noradrenaline. This coherence, therefore, gives us more confidence in the original observations, even though the explanation of both effects remains obscure.

**Do These Studies Have More Widespread Applicability?**

The opportunities offered by the uniqueness of the cardiac denervated heart preparation are manifold. Some important differences from the function of the normal innervated heart are highly relevant to the transplanted heart (e.g., the finding of similar abnormalities of glucose oxidation in the autotransplanted baboon heart and an adverse effect of chronic cardiac denervation on infarct size). With the progress of the transplantation programs it would be most interesting to correlate these findings with those in humans, as there is no evidence of reinnervation after nearly 2 years of transplantation.

**Summary**

Heart transplantation involves chronic effects due to denervation, rejection, and treatment of rejection. The chronically denervated dog heart provides a model for the effects of denervation alone. These hearts have been shown to contain intrinsic neurons with VIP and NPY as possible neurotransmitters. Myocardial tissue noradrenaline concentration falls to very low levels after degeneration of postganglionic sympathetic neurons, but dopamine remains in near-normal concentration and is probably synthesized extraneuronally. ANP is present and released normally; however, the natriuretic response to atrial distension is blunted, suggesting that this response is mainly due to a
reflex mechanism. Chronically denervated myocardial tissue exhibits increased oxygen consumption in vitro and increased Na-K-ATPase activity but has normal tissue levels of ATP and creatine phosphate. Glucose oxidation is inhibited in vivo, associated with increased levels of fructose-6-phosphate but normal glucose-6-phosphate, suggesting inhibition of phosphofructokinase activity. However, the enzyme protein concentration of phosphofructokinase, as judged by maximal in vitro activity, is normal. Maximal in vitro activities of succinate dehydrogenase, cytochrome oxidase, monoamine oxidase, calcium-dependent ATPase, and glycerolaldehyde-3-dehydrogenase are also normal. From these findings, we would predict that patients with transplanted hearts are likely to show myocardial metabolic inefficiency.

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