Choline acetyltransferase activity in rat heart after transplantation

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The parasympathetic innervation of the heart has two components. Preganglionic neurons, which originate in the dorsal motor nucleus of the brain stem, comprise the extrinsic parasympathetic innervation to the heart; postganglionic neurons, which originate and terminate within the heart, comprise the intrinsic parasympathetic innervation (6). During cardiac transplantation, the preganglionic neurons are severed; the postganglionic neurons remain intact (11, 14).

It would appear from previous work that reflex activation of the parasympathetic nervous system regulates heart rate and to a lesser extent the myocardial contractile state (6). Priola and co-workers (14) have reported that after surgical denervation of the heart the remaining intrinsic cholinergic neurons in the atria and the ventricles can be activated, and the magnitude of these responses are fairly uniform in the various chambers. This suggests that the distribution of the intrinsic parasympathetic innervation throughout the denervated heart may be uniform.

We therefore initiated studies to determine the pattern of the intrinsic parasympathetic innervation in the transplanted rat heart and the pattern of the combined extrinsic and intrinsic innervation in the intact heart by measuring the activity of choline acetyltransferase (CAT). CAT, which mediates the synthesis of acetylcholine, is postulated to be a marker of the parasympathetic innervation (15). Studies in this laboratory have demonstrated the presence of this enzyme intact innervated rat hearts, but not in myocardial cell cultures derived from this source (16).

If transplantation were to eliminate primarily the extrinsic innervation as a result of nerve sectioning, the remaining choline acetyltransferase activity would represent the intact intrinsic innervation (11). A difference in enzyme activity in normal and transplanted hearts could, therefore, represent an estimate of the extrinsic or preganglionic parasympathetic innervation.

MATERIALS AND METHODS

All studies were performed on male Sprague-Dawley rats weighing 200–250 g. Rats were given Purina rat chow and water ad libitum. They were anesthetized with chloral hydrate, 10 ml/kg (3.6% solution), by an intraperitoneal injection. Transplantation was carried out as reported previously (1, 3). Each recipient animal was prepared under ×16 magnification by a midline abdominal incision exposing the inferior vena cava and abdominal aorta distal to the renal vessels. The donor heart was removed by transecting the main pulmonary artery and the ascending aorta and ligating and dividing the vena cava and pulmonary veins. The heart was then placed in Ringer lactate at 4°C.

The donor heart was transplanted by joining the donor aorta to the abdominal aorta of the recipient and the donor pulmonary artery to the inferior vena cava of
the recipient. The interval between removal of the heart from the donor animal and its transplantation was less than 45 min. The anastomoses were created using x25 magnification and 10-0 monofilament nylon suture tipped with a BV-5 needle (Ethicon). Vascular occluding ties were released, permitting the delivery of oxygenated blood from the recipient's aorta through the donor coronary arteries. The pulmonary artery-cava anastomosis permitted blood from the coronary sinus, right atrium, and right ventricle to return to the recipient's circulation. A regular rhythm ensued in the donor heart after perfusion of the coronary arteries with oxygenated blood.

Pilot studies showed that choline acetyltransferase activity declined 70% in 2 days and was undetectable at 4 and 8 days in the distal portion of sectioned vagus nerves. Therefore, to provide time for the degeneration of preganglionic neurons, the animals were killed 8 days after transplantation. During this time, beating donor hearts could be palpated easily in the abdominal cavity of the recipient animal. Hearts maintained a regular rhythm and did not enlarge detectably.

The recipient rats were killed by spinal-cervical dislocation and tissues were obtained from the regions illustrated in Fig. 1. All samples were taken first from the donor heart and then from the normal host heart. Tissue samples were lightly blotted, weighed, immediately frozen in liquid nitrogen, and stored.

Choline acetyltransferase activity. To prepare stored tissues for assay of choline acetyltransferase, samples were disrupted in a tight-fitting stainless steel pulverizer cooled with liquid nitrogen (15-17). The powdered samples were further disrupted with a Teflon-to-glass, motor-driven, homogenizer in a minimum amount (less than 3 ml) of buffer (5 mM potassium phosphate, 0.1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4). The assay of acetylcholine biosynthesis in myocardial tissues was carried out as described previously (15-17, 19) by quantifying the formation of [Wlacetylcholine from [14C]acetlycoenzyme A.

A portion of the left ventricle was also taken for histological examination. Sections 6 µm thick were stained with hematoxylin and eosin for routine microscopic evaluation.

Statistical comparisons of mean values were made using Student's independent t test (20). A P value <0.05 was selected to denote statistical significance for all group comparisons.

RESULTS

Two of the eight transplanted hearts from animals that survived the operation were not beating when the animals were killed at 8 days; these were excluded from the study. The weights of the transplanted hearts (mean ± SE, 616 ± 34 mg) were not different from the weights of the recipients' hearts (605 ± 17 mg), nor was there a difference in the myocardial water content.

To investigate the possibility that the operation and storage might alter enzyme activity, hearts were assayed immediately after the animal was killed and after 45 min in Ringer lactate at 4°C. No change was noted in choline acetyltransferase activity after storage (Table 1).

Histological evaluation of the six beating transplanted hearts revealed that myocardial cells were in good condition, with only limited areas of ischemic changes. Focal areas of acute epicardial necrosis with minimal subepicardial changes were found, but these areas were limited to the subepicardial muscle; the myocardium and endocardium were normal. The morphology of the transplanted hearts did not differ from the host hearts or normal rat hearts (9).

Data on the cholinergic innervation and the regional distribution of CAT activity for host (control) and transplanted hearts is presented in Fig. 2. Control CAT activity was highest in the region of the sinoatrial node (30.8 nmol·min⁻¹·g⁻¹). Enzyme activity was high also in the right atrium and interatrial septum. Intermediate levels of enzyme activity were found in the left atrial appendage and in tissue near the conduction pathways, the superior interventricular septum, and the moderator band and the papillary muscle in the anterior right ventricle. Lower levels of CAT activity occurred in tissues more remote from the conduction pathways, including the inferior interventricular septum and the left ventricle.

In contrast to the host heart, which had nonuniform CAT activity, the transplanted hearts had uniformly low CAT activity in all the regions that were examined (Fig. 2).
To obtain an estimate of the sympathetic innervation, total catecholamine content was determined by a modification of the Anton-Sayre alumina-trihydroxyindole procedure (10). Mean values declined from 780 ± 80 ng·g⁻¹ in the normal hearts to 90 ± 10 ng·g⁻¹, indicating a substantial reduction of this neurotransmitter.

**DISCUSSION**

These studies demonstrate that choline acetyltransferase activity is distributed nonuniformly in the innervated host rat heart. The distribution of CAT activity in rat heart follows the same relative pattern as in the guinea pig heart (15, 17, 19) and is similar to the regional pattern of acetylcholine concentrations found in the rat (18) and the cat (2).

Surgical removal of the heart severs all nerves to it and results in degeneration of all fibers of extrinsic origin (preganglionic parasympathetic neurons). To document degeneration of the extrinsic (preganglionic) innervation, we confirmed that choline acetyltransferase activity became undetectable in the distal portion of sectioned vagus nerves in rats at 4 and 8 days after vagotomy (unpublished results). The intrinsic (postganglionic) neurons, on the other hand, should have remained intact (11).

In the present study, total myocardial denervation was confirmed by the marked decrease in the total catecholamine content. The small amount of residual catecholamines probably represents nonspecific binding of circulating catecholamines (4, 12). Furthermore, histological evidence suggested the myocardial cells were unaltered, yet the choline acetyltransferase activity decreased significantly.

The magnitude of our values should not have been changed by factors such as the operative procedure and temporary storage in Ringer lactate. No difference in enzyme activity was noted in hearts removed immediately and hearts that had been stored in 4°C Ringer lactate solution before transplantation. Thus, a reduction in enzyme activity did not result from the surgical procedure.

Myocardial alterations are an unlikely cause of the changes because no fiber atrophy was noted upon histological examination. In addition, no differences were found between the control and transplanted hearts. The transplanted heart does not function exactly like the normal heart in terms of cardiac output, but the left ventricle does eject small quantities of blood against a normal arterial systemic pressure. The working state thus is maintained. Although the work load of the transplanted hearts is decreased, 8 days in this state does not appear to be sufficient for detectable atrophy to develop.

Tissue rejection would not be expected since the donors and recipients were from identical strains (isographs). Transplanted (isograph) rat hearts similar to the present model have survived as long as 290 days with relatively normal electrocardiograms and no further histopathic changes (1). Therefore, from the normal histology, weights, water content, and the expected maintenance of a working state, we conclude that the hearts, although transplanted, remain viable and reasonably normal.

In previous studies, we have found choline acetyltransferase activity in intact innervated neonatal rat hearts, but not in myocardial cell tissue cultures derived from these hearts (16). The present data, along with the previous experiments, indicates that choline acetyltransferase has a neuronal rather than a myocardial origin.

The present data on choline acetyltransferase activity suggest a rather uniform postganglionic parasympathetic innervation throughout the transplanted heart. This result conforms with the recently published data on uniform functional responses to the activation of cholinergic neurons in denervated and transplanted canine hearts (14).

The differences in choline acetyltransferase activity between the transplanted and host rat hearts varied
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from region to region. This suggests strongly that the preganglionic parasympathetic innervation of the rat heart is nonuniform. In the atria, the greater than 90% decrease in choline acetyltransferase activity suggests that substantial enzyme activity may be associated with preganglionic neurons. In the left and right ventricles, on the other hand, the 50-60% decline in CAT after transplantation suggests that relatively less enzyme activity may have been associated with preganglionic fibers.

In agreement with our conclusion that the preganglionic parasympathetic innervation is nonuniform, Brown (2) found, in bilaterally vagotomized cats, a variable reduction in acetylcholine in the right atrium, sinoatrial node, papillary muscle, and right ventricle, but no change in left atrium, interventricular septum, or left ventricle. The minor differences between results of the present study and that of Brown (2) may be attributed to species specificity (rat vs. cat), methodology (transplant vs. bilateral vagotomy), or the component measured (choline acetyltransferase vs. acetylcholine).

We considered the possibility that the preganglionic parasympathetic innervation to the rat heart might exert a trophic influence on enzyme activity in the postganglionic neurons (13). If this were true, a portion of the observed reduction in CAT activity could have been associated with atrophic changes in the postganglionic innervation. Such a decrease would lead to an overestimation of the preganglionic component of the parasympathetic system. We cannot exclude this possibility in the rat heart. However, the possibility of trophic influences should not detract from the major conclusion that the preganglionic parasympathetic innervation to the rat heart is nonuniform.

It is possible that a large fraction of CAT in control hearts is derived from the intrinsic innervation that is maintained by a trophic influence of the preganglionic (extrinsic) innervation. If this is true, CAT activity after denervation (transplantation) might decline in the absence of a trophic influence from the extrinsic innervation. It is therefore possible that CAT activity in the intrinsic parasympathetic system of control hearts may differ from that observed in the transplanted hearts. We cannot exclude this possibility in the rat heart. In the transplanted hearts in this study, however, the interruption of extrinsic innervation and cessation of possible trophic influences produces a uniform pattern of CAT activity which presumably represents the nonstimulated postganglionic parasympathetic innervation.

In summary, these experiments demonstrate an intervention that successfully alters heart choline acetyltransferase activity in a predictable manner. Cardiac transplantation and earlier work in our laboratory indicate that choline acetyltransferase activity is a reliable marker for the parasympathetic innervation of the heart (16). The extrinsic parasympathetic innervation to the heart (preganglionic component) is nonuniform and supplies predominantly the specialized regions with conduction tissue. In contrast, the nonstimulated intrinsic innervation (postganglionic) is rather uniformly distributed in all the regions of the transplanted heart and comprises 50% or less of the total parasympathetic innervation of any heart region. These biochemical results are somewhat at variance with the reported absence of histological evidence for cholinergic ganglia in rat ventricles (7, 21). It is possible that the biochemical criterion of cholinergic innervation is the more sensitive. However, these results parallel the physiological findings that reflex activation of the parasympathetic system regulates heart rate and to a lesser extent the myocardial contractile state (5, 6, 8). Also, in the present study the direct evidence of a uniform postganglionic parasympathetic innervation to the heart parallels the physiological findings that activation of the intrinsic innervation pharmaco logically tends to produce uniform contractile responses in atria and ventricles (14).


