Return of neural responses after autotransplantation of the heart

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We have excised and reimplanted the heart in 25 dogs and have observed them for periods up to 30 months. Verification of the total extrinsic denervation and observations of immediate and early postoperative effects of this procedure have been reported elsewhere (16). Seven dogs with autotransplanted hearts have now survived longer than 1 year. We have obtained evidence in these long-term survivors that extrinsic neural connections (sympathetic, parasympathetic, and possibly sensory) have been re-established. These observations are the basis of this report.

METHODS

Cardiac excision and reimplantation (orthotopic autotransplantation) was performed by a technique described previously (15). This involves transection of the

venae cavae 1 cm from the right atrium, transection of the aorta and pulmonary artery 1 cm from the base of the heart, and transection of the left atrium just to the cardiac side of the confluence of the pulmonary veins. The excised, ischemic heart is protected by hypothermia at 20°C. Reattachment is accomplished by suture anastomosis of the cavae, aorta, pulmonary artery, and left atrium. The procedure is performed with the aid of extracorporeal circulation. The period of artificial circulation averaged 75 min and cardiac arrest varied between 40 and 60 min.

Nine of the twenty-five survivors were reoperated upon at intervals of 1 week to 24 months after the autotransplantation in order to determine whether reinervation had taken place. These procedures were performed under intravenous sodium thiopental (25 mg/kg) anesthesia. A segment of each vagus nerve was exposed in the neck, and the stellate ganglia were exposed through small thoracotomy incisions. A myocardial strain-gauge arch was sewn to the right ventricle for assessment of contractile force. A femoral or carotid artery was cannulated for measurement of systemic blood pressure with a Statham P23db transducer. Lead II of the electrocardiogram was monitored in several studies. Recordings were made with a multichannel direct-writing oscillograph. After the preparation had stabilized, the vagus nerves and stellate ganglia were stimulated electrically using bipolar electrodes. A Grass S-4 stimulator provided 1–30 stimuli/sec of 1–15 V and 0.3–10 msec duration.

In five of the above experiments, we recorded the cardiac chronotropic and inotropic responses and the systemic blood pressure response to intravenously administered tyramine (60 ìg/kg) and norepinephrine (0.25 ìg/kg). At other periods of observation, 9 of the 25 long-term survivors were tested with the same dose of norepinephrine while awake and restrained. During these observations the electrocardiogram was monitored in several studies. Recordings were made with a multichannel direct-writing oscillograph. After the preparation had stabilized, the vagus nerves and stellate ganglia were stimulated electrically using bipolar electrodes. A Grass S-4 stimulator provided 1–30 stimuli/sec of 1–15 V and 0.3–10 msec duration.

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In seven dogs surviving from 1 week up to 17 months...
we took biopsies of the left ventricle for measurement of catecholamine content. The excised tissue was rapidly frozen at the temperature of dry ice and analyzed by a fluorometric method (3).

The following were used as criteria for the presence of extracardiac innervation:

a) Prompt slowing of the heart after stimulation of the cervical vagus nerve (1).
b) Cardiac acceleration, augmentation, or both after stimulation of the stellate ganglion (1).
c) Cardiac acceleration, augmentation and elevation of blood pressure after infusion of tyramine (60 µg/kg) (2).
d) Increased blood pressure and cardiac augmentation without increase in rate after infusion of norepinephrine (0.25 µg/kg) (2, 4).
e) Bradycardia and hypotension in response to veratrum viride (16 µg/kg) (12).
f) Normal quantity of catecholamines in myocardial biopsies (3).

These were considered positive (+) responses. Absence of these features indicated lack of cardiac control by extrinsic nerves. Such observations were termed negative (−) responses.

RESULTS

Fifteen of the survivors were killed at intervals of 7–28 days for studies of neurochemical (11, 14) and morphological (13) features of the early phase of total extrinsic denervation. We have summarized in Table 1 the observations on dogs which were followed for longer periods of time. In no dog which had survived complete excision and reimplantation of the heart did we observe any evidence for connections with the nervous system before the lapse of 12 months after operation. Characteristic responses to vagus and stellate ganglion stimulation were absent. Tyramine injection gave no cardiac acceleration or augmentation. The catecholamine content of the myocardium was negligible. Norepinephrine injections were followed by unusual degrees of acceleration and augmentation. Infusions of veratrum viride alkaloids were not followed by bradycardia or hypotension.

At 12 months after cardiac autotransplantation, sinus arrhythmia began to appear regularly in electrocardiograms taken at rest, and changes in heart rate due to activity occurred more promptly. The dogs studied between the 12th and 24th month showed evidence for innervation in all the categories detailed above. Vagal stimulation stopped the heart and stellate ganglion stimulation resulted in prompt acceleration and augmentation even after decentralization. The effects of vagal stimulation were eliminated by the intravenous administration of hexamethonium bromide (2.5 mg/kg i.v.).

Tyramine injections were followed by cardiac acceleration and augmentation. The catecholamine content of the heart biopsies yielded normal values (0.50–0.60 µg/g wet wt.). Injection of norepinephrine (0.25 µg/kg i.v.) did not produce acceleration or an exaggerated augmentation response. The injection of veratrum alkaloids produced bradycardia and hypotension.

DISCUSSION

These observations indicate that the canine heart, after complete separation from all extracardiac attachments and replacement in its original position can sustain life for periods long enough to permit its reassociation with the central nervous system. The intrinsic adaptations required of the heart during its period of noninnervation are uncertain. However, it is clear that dogs with extrinsically denervated hearts can tolerate exercise (6), infusions (5), hypothermia (9), hemorrhage (9), anemia (7), and anoxia (8).

The precise time of reinnervation was not determined in a large series of animals because many were sacrificed for study in the early postoperative period or died from complications of the procedure. Reinnervation requires nearly a year, a remarkably long period when regeneration of autonomic nerves is considered to occur at a rate of 1 mm/day (10). Perhaps more remarkable is the fact that reinnervation occurs at all, since there is no division of extracardiac nervous trunks, and resumption of nervous connections must occur intramurally.

The way in which regenerative nerve fibers establish an effective relationship with other cardiac tissues is unsettled, as is the larger question of the finer relationship between nerve and muscle in the normal heart. One may speculate that residual postganglionic parasympathetic fibers and possibly some postganglionic adrenergic fibers (13) maintain an apparatus with which regenerating fibers establish a connection. Such a pathway could be afferent as well as efferent. Evidence that reinervating vagal fibers make contact with intramural ganglion cells rather than directly with effector cells was obtained by demonstrating that hexamethonium bromide (2.5 mg/
kg i.v.) abolished the bradycardiac response to stimulation of the vagus in the reinnervated heart.

The assumption that sensory reinnervation of the heart occurs is based on the return of the reaction of bradycardia and hypotension in response to injection of veratrum alkaloids. We have deferred more rigid tests of cardiac baroreceptor activity for fear of losing these animals during testing.

The dog with an autotransplanted heart is a valuable experimental preparation. Extrinsic denervation is unequivocally complete and prolonged, providing an unusual model for study of many fundamental properties of the heart. Moreover, when homologous transplantation of the heart becomes feasible from an immunological standpoint, these experiments support the view that the homotransplant will be capable of the adaptation necessary to support life in the noninnervated state while connections with the central nervous system are being re-established.

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