Electrophysiology of heterotopic heart transplant: Experimental study in dogs

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Electrophysiological properties were studied in a heterotopic heart transplant model developed in 44 dogs (Group I). Cycle length was 359.5 ± 55.2 ms in the recipient heart and 500.9 ± 77.9 ms in the donor heart (P < 0.001). Sinoatrial conduction time was 38.6 ± 13.6 ms in the recipient heart and 38.6 ± 10.4 ms in that of the donor (not significant). The Wenckebach point was 175.4 ± 31.1 ms in the recipient organ and 214.3 ± 42.6 ms in the donor heart (P < 0.001). The retrograde block point was 271.6 ± 48.0 ms in the recipient heart and 353.6 ± 47.3 ms in that of the donor (P < 0.01). The effective antegrade refractory period was 133.4 ± 28.7 ms in the recipient heart and 167.3 ± 46.3 ms in the donor organ (P < 0.001). An epicardial atrial cartography was performed at 44 preset points in both hearts, revealing a normal activation sequence and delays of 36–98 ms (mean 58 ms) in the recipient heart and from 39 to 59 ms (mean 50 ms) in the donor heart (not significant).

In another 11 dogs (Group II), the same electrophysiological study was carried out under basal conditions and after pharmacological blockade of the autonomic nervous system with atropine (0.04 mg (kg body weight)) and propranolol (0.2 mg (kg body weight)). No significant differences were found in any of the parameters studied upon comparison of Group II animals in basal conditions with Group I recipients, and Group II dogs after blockade with Group I donors.

These results demonstrate that the differences in automatism, conduction and refractoriness between the donor and recipient hearts are not attributable to differences in the haemodynamic situation or in the anaesthetic technique, but to denervation.

Introduction

While the electrophysiological properties of the innervated heart are well known[1], the same cannot be said for those of the denervated heart. Of the different techniques employed to study the denervated heart, each has its advantages and disadvantages. Pharmacological blockade of the autonomic nervous system is efficient[2–10] but transitory, and it can originate cross-reactions when other drugs are administered simultaneously. Surgical denervation without removal of the heart does not ensure the complete disconnection of the autonomic nervous system[2–10]; besides, the major drawback inherent in the study of the isolated heart is that the organ is not subjected to humorally mediated intrinsic influences.

In the orthotopically transplanted human heart, two electrophysiological peculiarities are produced: the complete denervation of the heart and the co-existence of two functioning sinus nodes. According to some authors, this circumstance represents a unique opportunity for electrophysiological and pharmacological studies[11]. The results, however, are variable and, in some cases, even contradictory[12–19]. One of the reasons for this is that the sinus node, aside from receiving deficient irrigation, is that of a terminally diseased heart[14,16]. Finally, since the recipient heart is removed, these reports only deal with sinus function, and not with the rest of the conduction system.

To date, we have found no reports on cardiac electrophysiology in transplants performed in animals, or descriptions of an experimental model that affords the simultaneous performance of these studies in a single experimental animal in order to compare the electrophysiological
characteristics of the denervated heart with the normal one.

The purpose of this study was to investigate the characteristics of automatism, refractoriness and conduction in a heterotopic heart transplant model in dogs, to compare the characteristics of the donor organ (denervated) with those of the recipient’s own heart, with a view to evaluating the influence of the autonomic nervous system on the conduction system of the two hearts in the same experimental animal.

Methods

Transplantation was performed in 44 dogs (Group I) of unknown age and breed, weighing between 25 and 30 kg. Donors were dogs whose body weight ranged between 15 and 20 kg. Care of the animals complied with that stipulated by the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals produced by the National Society for Medical Research and the National Academy of Sciences, respectively.

Anaesthesia was induced with sodium pentothal (20 mg (kg body weight)$^{-1}$). After endotracheal intubation, the dogs were connected to a Bird Mark 4 respirator and ventilated at a tidal volume of 15–20 ml (kg body weight)$^{-1}$. Anaesthetic maintenance was achieved with diazepam (5 mg after intubation), fentanyl (5–10 μg kg$^{-1}$ h$^{-1}$), pancuronium bromide (2 mg after intubation and 1 mg h$^{-1}$) and 30% oxygen and N₂O. Intravenous drug administration was discontinued 90 min prior to electrophysiological studies, in spite of which no spontaneous movements or alterations in electrocardiogram or arterial pressure were observed. In the recipients, the surface ECG, systemic and pulmonary arterial pressures, central venous pressure and pulmonary capillary wedge pressure were continuously monitored, as was cardiac output.

The surgical technique used for transplantation was that described by Zerbini’s group$^{17}$, in which the left chambers of the two hearts are connected in parallel (Fig. 1). The surgical approach in the donor was that of median sternotomy. Both venae cavae, the azygos vein, the supra-aortic trunks and the aorta, to the orifice of the first intercostal space, were dissected. After heparinization (3 mg (kg body weight)$^{-1}$), these vessels were ligated and the aorta was clamped. Cardiac arrest was induced with a cardioplegic solution at 4°C, introduced via the brachiocephalic trunk, and cooling was completed by irrigation with saline at 4°C in the pericardial sac. Once the organ was removed, the bridges of atrial tissue which separated the orifice of the left pulmonary veins were cut to connect them with the recipient atrium. The area of this orifice must be at least as large as that of the mitral valve of the donor organ. The right pulmonary veins were ligated. Then, the pulmonary artery was cut just before its bifurcation, as was the tip of the left atrial appendage; anastomosis between these structures was created to allow drainage of the coronary sinus since in this transplant model, the right cavities are excluded.

In the recipient, left thoracotomy was carried out and the pericardium cut widely open. The left atrial appendage and part of the atrium were isolated with an exclusion clamp. Then a longitudinal incision

\begin{figure}
\centering
\includegraphics[width=\textwidth]{diagram.png}
\caption{Diagram of the surgical technique. SVC: superior vena cava; IVC: inferior vena cava; RA: right atrium; LA: left atrium; RV: right ventricle; LV: left ventricle; PA: pulmonary artery; A: aorta.}
\end{figure}
was made to proceed to the anastomosis with the left atrium of the donor heart, the preparation of which was described previously. Then, the descending aorta of the recipient was clamped with a lateral exclusion clamp and, once the incision was made in this structure, a terminol-lateral anastomosis with the donor aorta was performed. The air was extracted from the chambers of the donor organ, the clamps were removed and heartbeat was restored by electric shock.

Once the haemodynamic situation stabilized and normothermia was achieved in the transplanted myocardium, fixed pentapolar reference electrodes were sutured into the right chambers of both hearts. They were connected to a computerized system based on microprocessors which integrated the signals, listed the results on a display, printed them, and plotted them on previously designed maps.

The signals obtained by reference electrodes were filtered between 50 and 500 Hz. With these signals, and by means of the system’s internal stimulator, programmed stimulation of the two hearts was achieved (5 v of amplitude and 1 ms of impulse width). The electrogram printout was obtained by interconnecting the system to an Electronics for Medicine VR-12 polygraph.

Cycle length (CL) was measured in the basal situation. Then, under stimulation, the following parameters were measured: sinoatrial conduction time (SACT) according to the method of Narula; A-V block points: antegrade or Wenckebach point (WP), and retrograde block point (RBP), by stimulation in atrium and ventricle, respectively, at CLs which decreased progressively by 50 ms; A-V effective refractory periods, antegrade (ARPAVN) and retrograde (RRPAVN), and effective ventricular refractory period (VRP), by stimulation at a fixed cycle and introduction of an extrastimulus with progressive reductions by 8 ms.

For the cartographic study, a map of the four atria was designed, in which the real surgical situation is reproduced with sufficient exactitude. Using a pentapolar electrode probe, the conduction delays were analysed at 44 predetermined atrial points in each heart. A second group of 11 animals (Group II) was anaesthetized with sodium pentothal and maintained exclusively with oxygen and N₂O after intubation. After median sternotomy, they were subjected to the same protocol of electrophysiological studies described for Group I, in the basal situation and after pharmacological blockade of the autonomic nervous system with atropine (0-04 mg kg⁻¹) and propranolol (0-2 mg kg⁻¹).

In the statistical analysis of the results, non-parametric tests were applied as the data obtained did not meet the criteria for normal distributions: Mann-Whitney U test for unmatched data in the comparison of two different hearts, and Wilcoxon’s t-test for matched data in the comparison of two different situations within the same heart.

Results

Basal heart rate of the conscious dogs employed varied between 85 and 110 beats min⁻¹, and after pentothal injection and thoracotomy increased to 156 ± 12 beats min⁻¹. This increase in the heart rate has been observed in association with i.v. injection of barbiturates, as well as with surgical aggression. The ischaemic times to which the donor hearts were subjected varied between 50 and 60 min. In no case was it necessary to resort to pharmacological support to recover or maintain the heartbeat.

After transplantation, both donor and recipient hearts maintained normal sinus rhythm, without atrial or ventricular arrhythmias, either spontaneous or induced. In both recipients and donors, the points of origin of the atrial activation were situated at the orifice of the superior vena cava, that is, in the sinus area (Fig. 2a). From there, the activation wave passed through the crista terminalis and toward the roof of the atrium via Bachmann’s bundle (Fig. 2b). The impulse then extended toward the free wall of both atria (Fig. 2c), and the last activation was situated in the region of the orifice of the pulmonary veins (Fig. 2d). In no case in this study were significant differences in the atrial activation delay detected between recipient hearts (36–98 ms; mean 58 ms) and donor hearts (39–59 ms; mean 50 ms).

With the exception of the SACT, the electrophysiological parameters studied were significantly different in the recipient and donor hearts, those of the denervated heart being more prolonged (Fig. 3).

The VRP could not be calculated in any of the donors because the QRS complex of these hearts did not produce a strong enough signal on the surface electrocardiogram and there was only a ventricular reference electrode available.

In group II, pharmacological blockade of the autonomic nervous system prolonged the CL, RBP and ARPAVN significantly (Fig. 4). Comparison of these results with those obtained in Group I revealed no statistically significant differences between the basal situation and that of the
transplant recipients to (Table 1). Likewise, no statistically significant differences were found between the studies of the donor hearts and those of the animals subjected to pharmacological blockade of the autonomic nervous system (Table 1). The differences between the recipient hearts in Group I and Group II hearts subjected to pharmacological blockade overlap with those found between the recipient and the donor (Fig. 3), except with respect to the WP. It could be that the number of observations in Group II was insufficient for the determination of this parameter.

Likewise, the differences between the donors in Group I and basal situation in Group II are within the same range as that resulting from the comparison of the basal situation and post-pharmacological blockade in Group II (Fig. 4).

It is worth noting that of the 44 transplantations reported here, only 12 recipient hearts and 11 donor organs demonstrated retrograde conduction. Of the 11 animals subjected to pharmacological blockade, in only four was there retrograde conduction.

**Discussion**

It has been reported that the orthotopically or heterotopically transplanted human heart generally has a faster rate than the normal innervated heart[12-14,16-22]. This has been observed in early and late postoperative periods[23,24], but there is no mention of this phenomenon during surgery.

The increase in heart rate has been attributed to the abolition, by denervation, of the predominant vagal influence in physiological situations[2,4,22,23] and to the fact that any denervated organ becomes much more sensitive to its usual neurotransmitter[26,27].

The explanation, however, should not be so simple since, although the rate of the transplanted heart coincides with that obtained with pharmacological blockade of the autonomic nervous system[5,4,5], with this same technique it has also been demonstrated that in 40% of healthy individuals, the predominance is not vagal, but sympathetic[29] and that the intrinsic heart rate is age-dependent[39].
This same chemical denervation performed in dogs has revealed that the modifications of heart rate also depend on whether the animals are subjected to general anaesthesia and whether thoracotomy is carried out\(^6\).

In the conscious animal, pharmacological blockade has practically no effect on heart rate; when the animal is anaesthetized, the rate increases; and in anaesthetized dogs subjected to thoracotomy, it decreases by approximately 20%.

This is due to the fact that surgery induces a sympathetic predominance which is suppressed by the blockade. In all three situations, the intrinsic heart rate is the same\(^6\).

Our data regarding CL coincide with the findings of other authors who observed that in anaesthetized and thoracotomized dogs, pharmacological blockade induces a 20% decrease in heart rate\(^6\).

Compared with other reports, the CLs of the transplanted hearts in this study are within the same range, and remain constant for 3–4 days until the slowing characteristic of rejection appears\(^28\).

Our data on heart rate differ substantially from those obtained using other surgical denervation techniques not entailing heart removal\(^37\).

In these reports, the CLs of the supposedly denervated
hearts correspond to those of the recipients in our study, but not those of the donors. It is known that with these techniques sympathetic disconnection is incomplete.

The autonomic nervous system affects A-V conduction much in the same way as it affects sinus automatisma: the sympathetic stimulation or predominance shortens the intervals of A-V conduction, while vagal stimulation or predominance lengthens themb. Our finding of longer WP, RBP and ARPAVN in the donor than in the recipient may also be an expression of the suppression of the sympathetic predominance originated by denervation.

According to numerous anatomical and physiological studies, the SN and A-V nodes are the zones with the greatest concentration of adrenergic and cholinergic fibres. Therefore, it seems reasonable to assume that denervation would affect fundamentally the function of these structures. This hypothesis is confirmed by the results of our study in that sinus automatisma and A-V conduction are modified in donor hearts, while SACT and atrial activation delays showed no differences with respect to the recipient hearts.

Regarding intra-atrial conduction, we have detected no influence of the autonomic nervous system since there were no differences between recipient and donor hearts in activation delay.

Several factors related to the surgical technique—hypothermia, ischaemia, physical distortion, etc. — can alter the conduction of the impulse from the SN to the A-V union. The integrity of the atrial conduction of donor hearts in our study demonstrates that the experimental model employed produces no severe atrial lesions. Another advantage of this model is the possibility of comparing a denervated heart with another normal heart simultaneously and in the same laboratory animal. This obviates the inconveniences of electrophysiological studies in orthotopic transplantation, in which the absence of a complete recipient heart limits the studies exclusively to the function of the recipient SN, a structure which does not receive normal irrigation and has been subjected to ischaemia during the surgical procedure.

The auxiliary heterotopic heart transplant utilized by our group makes it possible to carry out complete, simultaneous electrophysiological studies, and does not require the use of extracorporeal circulation, thus avoiding the induction of alterations in the recipient which might produce artifactual results.

The principal criticism that can be made of this model is that the hemodynamic situations of the recipient and donor hearts are different, which could cause differences in the results of the electrophysiological studies. However, we have proved that this is not so: in anaesthetized and sternotomized animals (Group II), the results of the electrophysiological study prior to blockade were identical to those of the transplant recipients. After blockade of the autonomic nervous system with atropine and propranolol, the results were superimposable on those of the donor hearts. These findings demonstrate that the different hemodynamic situations have not affected the results of the electrophysiological study. The only exception, and thus the only doubt, concerns the WP: this parameter is significantly different only when the comparison is made between recipient and donor. Taking the results together, this finding could be due to an insufficient number of observations in Group II with respect to this parameter.

We consider that this heterotopic heart transplantation model may be useful in the study of the effects of different pharmacological agents on the conduction system of the transplanted heart (denervated), and will help us learn whether the mechanism of action is direct, or mediated by the
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