CHANGES IN ELECTRICAL ACTIVITY OF THE MYOCARDIAL FIBERS AFTER SUBCUTANEOUS TRANSPLANTATION OF THE HEART INTO THE MOUSE EAR

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The intracellular resting potential (RP) and the action potential (AP) of the transplanted mouse heart on the third to fourth day after transplantation were lower than the intracellular potentials of newborn mice. Starting from the fifth to sixth day there was a gradual increase in the amplitude of the intracellular potentials. On the first 7-8 days after isologous and heterologous transplantation the dynamics of the changes in intracellular activity was the same. Later, in the case of the heterograft, a second decrease in amplitude of RP and AP of the myocardial fibers of the graft took place, probably in connection with the development of a rejection reaction. In the isografts the amplitude of the intracellular potentials toward the end of the first month after transplantation was close to the values of RP and AP of the fibers of the recipient's heart.

KEY WORDS: *transplantation of mouse heart; microelectrodes.*

After the development of a technique for transplantation of the newborn mouse heart subcutaneously into the ear of adult mice in 1963 [1] this model has become widely used in experimental immunology for the study of various problems in transplantation. Under these conditions it is possible to study the properties of denervated heart tissue functioning *in vivo*. The viability of the graft can be assessed from the amplitude of the electrical activity of the grafted heart [2, 3], but this gives no idea of changes in the individual myocardial cells.

The object of this investigation was to study changes in the intracellular potentials of the myocardial fibers of the graft and of the recipient's heart (in connection with the possibility of development of a "graft versus host" reaction).

EXPERIMENTAL METHOD

The method of cardiac transplantation was fully described previously [1]. Starting from the 3rd-4th day after transplantation, the ECG was recorded daily in each animal in three standard leads, and the electrical activity of the heart transplanted subcutaneously into the ear also was recorded. Mice weighing 20-25 g were given an intraperitoneal injection of 0.1-0.12 ml of thalamonal (standard solution in ampuls). The animals were stretched out on a transparent plastic plate and the head was fixed between two corks, so that two metallic electrodes could easily be introduced into the ear, along diametrically opposite edges of the graft. Anesthesia continued for 15-20 min. Everyday, starting from the 3rd day after transplantation, one mouse was used in an acute experiment. The graft was separated from the ear and placed in an experimental chamber through which Tyrode's solution for heart muscle of warm-blood animals, aerated with carbogen (96% O_2 + 4% CO_2) circulated. The auricle of the recipient's heart was placed in the same chamber. Intracellular potentials were recorded by glass microelectrodes filled with 3 M KCl solution. Altogether 150 mice were studied (three on each day after transplantation).

EXPERIMENTAL RESULTS

In order to draw a line between the changes caused by the development of immunological conflict and taking place in the postoperative period, and those unconnected with tissue in-

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TABLE 1. Amplitude of ECG and Electrical Activity of Graft (in mV) in Period of Transplantation (M ± m)

Day after transplantation	Recipient (ECG)			Graft (electrical activity)		
	serial no. of mouse					
	Isografting					
	2	12	15	2′	12′	15′
4-th	1,8±0,1	0,8±0,05		0,24±0,03	0,19±0,02	0,2±0,01
5-"	0,55±0,05	1,3±0,1	1,3±0,1	0,25±0,02		0,2±0,01
6-"	1,4±0,1		1,4±0,1	0,27±0,03		0,5±0,05
7-"	1,4±0,1	1,05±0,1	1,3±0,1		0,45±0,07	
8-"		0,95±0,05			0,16±0,02	
10-"			1,2±0,1			0,25±0,05
11-"	0,9±0,1		1,35±0,5	0,8±0,0		0,2±0,01
16-"			0,5±0,05			0,4±0,05
17-"			0,7±0,5			0,4±0,05
18-"	0,9±0,05		0,7±0,05	0,42±0,02		0,65±0,05
19-"	0,75±0,05		0,9±0,1			0,7±0,1
20-"	0,35±0,01		0,9±0,1	0,11±0,02		0,7±0,1
21-st			0,4±0,1			0,8±0,1
24-th			1,1±0,1			1,0±0,1
31-st			1,3±0,1			1,0±0,1
	Heterografting					
	1	5	10	1′	5′	10′
3-rd			0,27±0,03			0,12±0,02
4-th	0,55±0,05	0,5±0,05	0,55±0,05	0,12±0,01	0,15±0,01	0,11±0,01
5-"	0,6±0,05	0,3±0,0	0,5±0,05	0,21±0,01	0,29±0,01	0,3±0,02
6-"	0,5±0,05	0,35±0,05	0,3±0,1	0,5±0,01	0,3±0,01	0,32±0,02
7-"	0,5±0,05	0,15±0,03	0,33±0,02	0,55±0,05	0,23±0,01	0,11±0,01
8-"	0,6±0,05					
9-"	0,82±0,04			0,21±0,01		

compatibility, electrical activity of the recipient's heart was compared with that of the graft after isologous and heterologous transplantation.

The amplitude of the QRS wave of the recipient's ECG and of electrical activity of the graft (mean of 10 measurements) for three mice after isologous transplantation, from the 4th to the 31st day after grafting is shown in Table 1 (top part). The amplitude of the QRS wave of the recipient's heart varied within wide limits and showed no definite correlation with the time after transplantation. The amplitude of electrical activity in the graft varied considerably on different days. Only in a few animals (in mouse No. 15, for example) could a gradual increase in its amplitude be observed in the course of a month without any sharp falls.

In the case of heterologous grafts (see the bottom part of Table 1), just as of isologous, no correlation could be found between the amplitude of the QRS wave and the time after transplantation in the recipient's heart. The amplitude of electrical activity of the allografts, like that of the isografts, increased on the 4th-5th day after transplantation. The maximum occurred on the sixth to seventh day. The amplitude of electrical activity of the graft then fell again, evidently because of the development of a rejection crisis, for thereafter the graft ceased to function.

Intracellular recording of the potentials (Table 2) showed that the resting potential (RP) and the amplitude of the action potential (AP) of the myocardial fibers of the recipient's heart were unchanged in the course of transplantation (Table 2, mean results of 10 measurements). Intracellular potentials of an isograft of the atrium (auricle) are shown in Fig. 1. Clearly on the 3rd day after transplantation (frame C) the fiber was depolarized. In response to application of a stimulus low-amplitude double responses appeared, with a low initial rate of rise. By the seventh to eighth day (frames D and E) the amplitude of RP and AP had increased, and their rates of rise were also much greater; as a rule at this time the specimens contracted spontaneously. In the later stages of transplantation (frame F) RP and AP of the fiber were greater than initially (frame A).

After heterografting the amplitude of the intracellular potentials of the recipient's heart was either unchanged (Table 2, group 1) or reduced (group 2, RP column) compared with

TABLE 2. Intracellular Potentials (in mV) During Period of Transplantation (M ± m)

Day after transplantation	Atrium recipient		Ventricle graft			
	Isografting					
	RP	AP	RP	AP	RP	AP
4- th	92,8±2,1	119,8±2,1			46,2±1,4	55,4±3,3
5- "	91,1±3,34	120,9±3,7	52,9±4,4	65,5±6,2	44,2±7,6	34,4±6.8
6- "	74,6±2,8	103,5±1,9	73,3±4,7	77,9±3,2		
7- "	91,2±1,2	121,4±0,7			67±3,6	71,3±2,1
11- "			81±1,6	107±1,3	54.8±11,3	74,7±8,4
24- "	85,9±1,7	114±1,2			70,7±0,88	90,6±2,5
27- "	86,8±1,2	109±1,4	77,4±0,72	100,6±0,7	89,6±1,8	113±2,2
29- "			85,3±2,6	95,1±3,2		
33- rd	90,8±0,53	113,4±1,8	81,4±0,89	102±1,1		
34- th	90,2±3,6	119±2,7	81,4±2,8	93,1±0,76		
36- "	91,8±1	103,8±1,8			80,4±5,5	98,5±3,9
	Heterografting Group 1					
5- th	88,4±0,64	98,8±0,89			61,4±2	55,8±0.33
6- "	95,6±1	109,5±3,5	56,4±2,1	71,6±1,64	49±2,9	51±1
7- "	85±3,3	96,3±4,1	76±2,2	88,6±0,89		
8- "	83,7±3,1	107±10,3	67,8±3,6	76±3,7		
9- "	85,5±3,2	92,6±1,5	80±1,5	91,7±0,6		
10- "	88,7±0,65	119,7±0,86	62,1±1,1	80,7±0,6		
	Group 2					
3- rd			40,3±10,3	43,6±13,6	55±4,6	55,8±4.7
4- th	68,2±3,1	87,9±2,7	66,7±2,7	72,7±1		
5- "	74,9±3,1	97,7±6,5	60,7±4,1	73±0,74	75,2±3,1	85,7±4
6- "	76,2±1,7	95,2±2	69,9±3,5	79,7±2,5	47,5±9	63,3±2.1
7- "	78,5±3,6	101,4±3,8			75,4±5,5	91,7±1,9
10- "	78,9±3,8	110±8,9			62,7±1,8	56,3±1,2

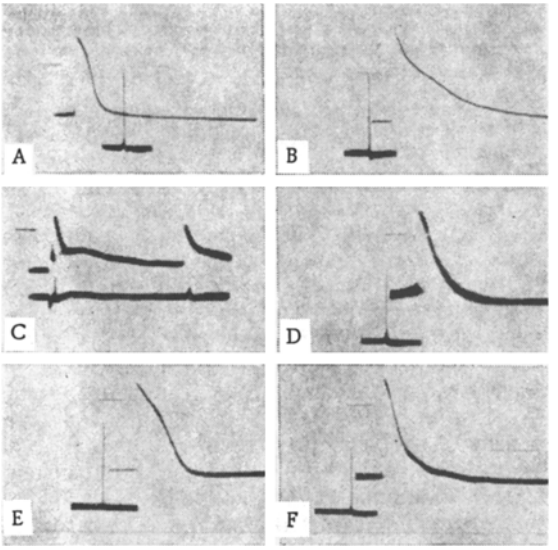

Fig. 1. Action potentials of atrial cells in isografts. A) Newborn mouse, 3 days; B) mother's heart; C,D,E,F) graft on 3rd, 7th, 8th, and 34th days respectively after transplantation; horizontal line on left of AP in each frame gives level of zero potential; curve below is first derivative of AP. Calibration: vertically, 50 mV and 40 V/sec; horizontally, 40 msec.

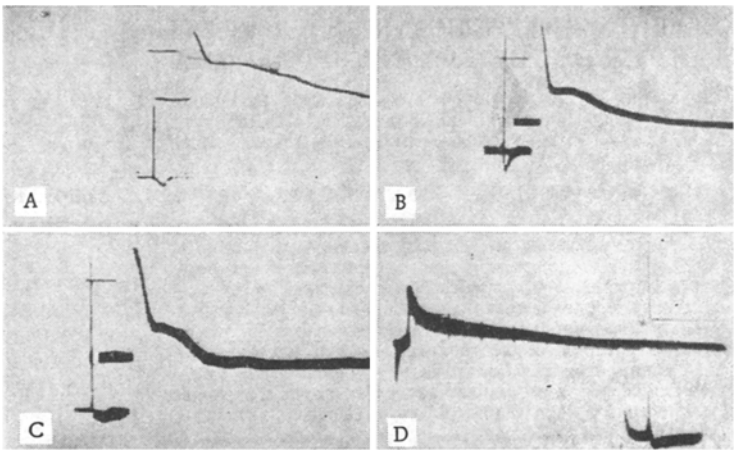

Fig. 2. Action potentials of atrial cells in heterografts. A,B,C,D) graft on 3rd, 4th, 5th, and 13th days respectively after transplantation; remainder of legend as in Fig. 1.

its level after isologous grafting. The dynamics of the change in RP and AP of the myocardial fibers of the atrial heterograft is shown in Fig.2. Initially, the intracellular potentials of the graft from newborn mice were the same as in isografts (Fig. 1, frame A). The amplitude of RP and AP was reduced 3 days after transplantation. The AP was considerably lengthened and a plateau phase appeared (frame A). Starting from the fourth to fifth day a well-defined AP with a rapid rate of rise was formed (frames B and C). On the 13th day after transplantation the amplitudes of RP and AP of the fibers fell sharply, evidently in connection with the development of a graft rejection reaction.

It was shown previously [3] that on the second to third day after transplantation the graft is in a state of ischemia and inflammation. Starting from the 4th day, new blood vessels formed intensively. After isologous transplantation the graft could contract spontaneously for up to 1 year and it died as a result of proliferation of connective tissue beneath the skin of the ear and not on account of immunological conflict. In the case of heterologous grafting, the course for the first 6 or 7 days after transplantation was similar to that of isografting; not until the ninth or tenth day (or later, depending on the degree of difference between the donor and recipient with respect to HL-A antigens) did a rejection reaction develop.

This investigation showed that in the course of transplantation the amplitudes of the ECG of the recipient's heart and of the electrical activity of the graft changed in both heterologous and isologous mice; considerable caution must be exercised when the ECG and electrical activity of the graft are used as criteria of rejection in the case of heterografting of the heart.

By microelectrode investigations it was possible to record high-amplitude electrical activity of the myocardial fibers of the recipient's heart and of the graft. Despite the fact that the mean values of the RP and AP indices were obtained in different animals, they differed only a little from one another for the recipient's heart after isografting. The decrease in RP in group 2 (Table 2) after heterologous transplantation may perhaps be due to the influence of the graft on the recipient's heart. By microelectrode recording it was possible to study the dynamics of changes in the electrical activity of individual heart cells of the graft in the course of transplantation. Together with the special conclusions, one general conclusion can also be drawn: the isografted mouse heart can be used as a convenient model of denervated cardiac cells functioning parallel with the recipient's intact heart.

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SEGMENTAL RECIPROCAL REFLEXES OF THE THORACIC PORTION OF THE SPINAL CORD

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Under ordinary experimental conditions stimulation of the central end of a divided intercostal nerve causes reflex discharges simultaneously in several intercostal nerves on the same and opposite sides of the chest. No reciprocity is observed between these reflexes. It is shown as a result of this investigation that mechanical stimulation of the parietal pleura of unanesthetized spinal cats facilitates reflex discharges in the intercostal nerves on the side of stimulation of the pleura and inhibits them on the opposite side. The presence of reciprocal segmental reflexes between the left and right halves of the chest was thus established for the first time.

KEY WORDS: *respiratory muscles; intercostal nerves; polysynaptic reflexes; pleura; reciprocal innervation.*

It has been known since Sherrington's time that the lumbosacral and cervical segments of the spinal cord have mechanisms which provide reciprocal relations between the homonymous muscles of the opposite limbs. At the thoracic level of the spinal cord no such mechanisms have been found: stimulation of the central end of a divided intercostal nerve evokes reflex discharges simultaneously in several intercostal nerves on the same and opposite sides of the chest [7]. This corresponds to the fact that the two halves of the chest function in phase during respiration.

Besides their role in ventilation of the lungs, the respiratory muscles also participate in the maintenance of posture. During the performance of this function, reciprocal relations are observed between the respiratory muscles of the left and right sides of the chest. Usually this reciprocity is ascribed to descending influences from the cerebellum and the receptors of the neck [1, 5, 9, 10]. However, certain clinical observations suggest that reciprocity between the respiratory muscles of the left and right sides of the chest may also be due to segmental mechanisms in the thoracic part of the spinal cord. A well known example of such observations is the restriction of respiratory movements of the chest on the side of pleurisy. It seems likely that this asymmetry of the respiratory movements is caused by sensory impulses arising in the inflamed pleura and exerting a reciprocal effect on the reflex mechanisms of the left and right sides of the thoracic segments of the spinal cord.

The investigation described below was undertaken to study this problem. The effect of mechanical stimulation of the parietal pleura on reflex discharges evoked in the intercostal nerves by stimulation of neighboring intercostal nerves was studied.

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