EFFECTS OF ISOPROTERENOL ON THE DEVELOPING HEART IN RATS

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Many clinical clues suggest a link between abnormal catecholamine function during a phase of rapid cardiac development and hypertrophic cardiomyopathy. However, experimental investigation focusing on abnormal catecholamine function during the fetal period as the pathogenesis of hypertrophic cardiomyopathy has not yet been carried out. In this study, isoproterenol (ISO) was administered to pregnant female rats and the effects of the drug on the hearts of their offspring were studied morphologically.

Fifty $\mu\text{g/kg/day}$ of ISO was administered subcutaneously to 15 pregnant female rats 5 days a week for 3 weeks and the offspring were killed at 2 days, 4 weeks or 7 weeks after birth. Isotonic saline was administered to 15 pregnant female rats as control. The hearts of the offspring were removed and weighed immediately. The ventricles were cut in two, parallel to the atrio-ventricular groove. Left and right ventricular free wall thickness and interventricular septal thickness were measured. Under light-microscope, myocardial fiber diameter was measured and myocardial fiber disarray was assessed. Fine structural alterations of the cardiac muscle cells were observed by an electron microscope.

The hearts of newborn offspring from pregnant rats treated with ISO showed disproportionate septal hypertrophy and frequent inter- and intra-cellular disarray in the interventricular septum. However, these changes were not prominent at 7 weeks after birth. These results suggest that abnormal catecholamine function during the fetal period may result in disproportionate septal hypertrophy.

IT is well known that hypertrophic cardiomyopathy frequently occurs in association with disorders of structures derived embryologically from the neural crest, including pheochromocytoma, lentiginosis or Friedreich's ataxia.¹⁻⁴ A number of clinical clues suggest a link between abnormal catecholamine function during a phase of rapid cardiac development and hypertrophic cardiomyopathy.⁵ Olsen reported that widespread myofibrillar disarray occurs in the offspring of rats treated with triiodothyroacetic acid

(triac) during pregnancy and proposed a possible relationship between hypertrophic cardiomyopathy and hyperthyroidism.⁶⁻⁸ However, experimental investigation focusing on abnormal adrenergic function during the fetal period as a pathogenesis of hypertrophic cardiomyopathy has not yet been carried out.

In order to ascertain the role of abnormal catecholamine function in the pathogenesis of hypertrophic cardiomyopathy, isoproterenol, which readily transports through the rat placenta and leads to tachycardia in the embryonic rat heart⁹ was administered to pregnant female rats and the effects of the drug on the hearts of their offspring were studied morphologically.

Key words:

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TABLE I THE NUMBER OF FEMALE RATS AND THE PREGNANCY RATE

	Number	No. pregnant	Average litter size
Isoproterenol	15	9	10.3
Control	15	9	10.8

Isoproterenol: 50 µg/kg/day of isoproterenol injection

Control: 0.2 ml of saline injection

TABLE II THE NUMBER OF MALE OFFSPRING AND THE TIME LAPSE OF SACRIFICE AFTER DELIVERY

	2 days	4 weeks	7 weeks
Offspring from isoproterenol treated rat	23	14	10
Control offspring	20	15	10

MATERIALS AND METHODS

Thirty virgin female Wistar rats, average weight 200g were divided into 2 groups of 15 rats (Table I). All rats were fed a stock chow diet (NMF, Oriental Yeast Co., Ltd) and tap water ad libitum throughout the experiment. On day 1 the rats were caged individually, each with a male of the same strain. On day 8 the males were removed. The pregnancy-rate is shown in Table I. L-isoproterenol (Sigma, 50 µg/kg/day) dissolved in isotonic saline was administered subcutaneously to the female rats for 5 days a week from days 1–19 (i.e., 15 injections each). Control animals received equal volumes of isotonic saline solution without isoproterenol. All litters were born after 24–27 days. The male offspring were killed at 2 days, 4 weeks or at 7 weeks after delivery. The number of animals and the time lapses after delivery in the respective group are given in Table II. Immediately after sacrifice, the beating hearts were removed and immersed in 2.5% phosphate-buffered glutaraldehyde solution. After a few seconds, the heart ceased beating in a more or less diastolic state.¹⁰ The ventricles were cut into two, parallel to the atrio-ventricular groove and weighed. The ventricles of the newborn were fixed with 2.5% phosphate-buffered glutaraldehyde solution for 3 hrs at 4°C. They were post-fixed in 1% osmium tetroxide, dehydrated with an ascending series

Fig.1. Wall thickness was measured at three portions excluding the papillary muscle and the trabeculae and the average thickness of the three portions was calculated.

of ethanol and embedded in Epoxy resin. The ventricles of the offspring, 4 and 7 weeks old, were fixed with 10% phosphate buffered formaldehyde solution, dehydrated with ethanol and embedded in paraffin. An entire cross-section 2 or 8 µm thick was stained with alkaline toluidine blue or hematoxylin-eosin.

Left and right ventricular free wall thickness and interventricular septal thickness were measured on micrographs of the sections at ×20 to ×60 magnification. Wall thickness was measured at three portions, excluding the papillary muscle and the trabeculae, and the average thickness of the three portions was calculated (Fig. 1). Then, the septal to left ventricular free-wall thickness ratio was calculated. In order to determine the muscle fiber diameters, the shortest transverse diameters of fibers crossing through the nuclei in the inner one-third of the wall were measured at 800 times magnification in 200 consecutive muscle fibers.

Disarray of myofibers was graded (0) to (3+) depending upon the severity of cardiac muscle cell disorganization. Grade (0) signifies no apparent fiber disarray; (1+) moderate; (2+) marked; (3+) excessively marked fiber disarray (Fig. 3 A-D), and the fiber disorganization in the free wall and the septum were assessed. Ultrathin sections from the remaining apical portion were stained with uranyl acetate and lead citrate and examined with an electron microscope.

Fig.2. Horizontally sectioned ventricles of newborn rats. (Scale bar = 1 mm).
 (A) The ventricle of newborn from the mother treated with isoproterenol shows disproportionate septal hypertrophy.
 (B) The ventricle of the control newborn.

Fig.3. Histologic sections of the ventricular septum from the control newborn rats (A, B) and the isoproterenol-treated newborn (C, D). (A): Grade (0). (B): Grade (1+). (C): Grade (2+). (D): Grade (3+). (Alkaline toluidine blue stain. $\times 220$.)

Differences between means were tested by unpaired Student's t-test. Chi-square test or Fisher's exact probability test was used to compare the severity of fiber disorganization. Statistical significance was defined as $p < 0.05$. All values are expressed as the mean $\pm$ S.D.

RESULTS

Newborn Rats

Body weight, ventricular weight and ventricular weight to body weight ratio at sacrifice are summarized in Table III. There was no signifi-

Fig.4 (A) Electron micrograph of a newborn rat from a control group. Myofibrils are predominantly regularly arranged. (B, C) Electron micrographs of a newborn rat from isoproterenol-treated mother. Severe disarray of myofibrils and myofilaments are noted. (D) Electron micrograph of a control newborn rat. Disarrangement of myofibrils and myofilaments are noted near the intercalated discs. (Lead citrate and uranyl acetate. Scale bar = 1 μ m.)

TABLE III BODY WEIGHT, VENTRICULAR WEIGHT AND VENTRICULAR WEIGHT/BODY WEIGHT RATIO

		Body weight (g)	Ventricular weight (mg)	Ventricular weight/body weight ratio
2 days	Isoproterenol	6.7 $\pm$ 0.4	21.7 $\pm$ 1.7**	3.23 $\pm$ 0.19**
	Control	6.8 $\pm$ 0.4	19.7 $\pm$ 3.0	2.91 $\pm$ 0.37
4 weeks	Isoproterenol	51.6 $\pm$ 4.3	241.5 $\pm$ 28.2*	4.68 $\pm$ 0.42**
	Control	52.6 $\pm$ 4.9	217.7 $\pm$ 17.2	4.15 $\pm$ 0.30
7 weeks	Isoproterenol	163.5 $\pm$ 15.7	518.5 $\pm$ 45.2*	3.18 $\pm$ 0.18
	Control	150.9 $\pm$ 20.0	462.2 $\pm$ 57.3	3.06 $\pm$ 0.13

Values are expressed as mean $\pm$ SD. **: $p < 0.01$; *: $p < 0.05$

cant difference in body weight while significant ventricular hypertrophy appeared in the offspring from the mother rats treated with isoproterenol. Ventricular free wall thickness, interventricular septal thickness and septal-free wall thickness ratio are given in Table IV. Although there was

no significant difference in the left ventricular free wall thickness, the ventricular septum of the offspring from the mothers treated with isoproterenol was significantly thicker than that of the control. The mean septal-free wall thickness ratio of the newborn treated with iso-

TABLE IV VENTRICULAR SEPTAL, LEFT AND RIGHT VENTRICULAR FREE WALL THICKNESS AND VENTRICULAR SEPTAL TO LEFT VENTRICULAR FREE WALL THICKNESS RATIO

		Wall thickness (mm)			
		Ventricular Septum	Left ventricular free wall	Right ventricular free wall	Septal-LV free wall ratio
2 days	Isoproterenol	0.622 ± 0.078**	0.572 ± 0.077	0.339 ± 0.058	1.09 ± 0.13**
	Control	0.503 ± 0.079	0.558 ± 0.082	0.326 ± 0.060	0.90 ± 0.07
4 weeks	Isoproterenol	1.38 ± 0.11	1.61 ± 0.11	0.76 ± 0.10	0.86 ± 0.07
	Control	1.24 ± 0.11	1.50 ± 0.19	0.74 ± 0.08	0.83 ± 0.07
7 weeks	Isoproterenol	1.60 ± 0.21	1.86 ± 0.15	0.83 ± 0.09	0.86 ± 0.09
	Control	1.48 ± 0.11	1.80 ± 0.17	0.87 ± 0.10	0.83 ± 0.08

Values are expressed as mean ± SD. **: $p < 0.01$; *: $p < 0.05$

TABLE V FIBER DIAMETER IN THE SEPTUM AND THE LEFT AND RIGHT VENTRICLES

		Fiber diameter (μm)		
		Ventricular Septum	Left ventricular free wall	Right ventricular free wall
2 days	Isoproterenol	8.2 ± 1.0**	8.4 ± 0.9**	8.1 ± 1.1**
	Control	7.6 ± 0.6	7.6 ± 0.9	7.5 ± 0.8
4 weeks	Isoproterenol	11.1 ± 1.1**	10.7 ± 1.1*	10.7 ± 1.1*
	Control	10.1 ± 1.2	10.3 ± 1.4	10.2 ± 1.1
7 weeks	Isoproterenol	12.5 ± 1.4*	12.3 ± 1.6	11.8 ± 1.4
	Control	11.8 ± 1.7	12.0 ± 1.8	11.6 ± 1.3

Values are expressed as mean ± SD. **: $p < 0.01$; *: $p < 0.05$

TABLE VI GRADING OF FIBER DISORGANIZATION

		Ventricular septum			Left ventricular free wall		
		grade 0-1+	grade 2+-3+	p-value	grade 0-1+	grade 2+-3+	p-value
2 days	Isoproterenol	8	15	< 0.01	19	4	NS
	Control	16	4		19	1	
4 weeks	Isoproterenol	12	2	NS	14	0	NS
	Control	14	1		15	0	
7 weeks	Isoproterenol	10	0	NS	10	0	NS
	Control	10	0		10	0	

NS = not significant

proterenol was 1.09 ± 0.13 which was significantly greater than that of the control (0.90 ± 0.07) (Table IV and Fig. 2). No significant

difference was observed in the right ventricular free wall thickness. Fiber diameters were significantly larger in isoproterenol treated animals both

Fig.5. Horizontally sectioned ventricles of 7-week-old rats. (Scale bar = 5 mm.)
 (A) The ventricles of the offspring of the mothers treated with isoproterenol showed that left ventricular free wall and septum are of similar thickness.
 (B) The ventricles of the control offspring.

Fig.6. Histologic sections of the ventricular septum from the 7-week-old offspring. Cardiac muscle cells in an isoproterenol-treated (A) and a control offspring (B) are predominantly arranged in parallel (Hematoxylin and eosin stain. $\times 220$.).

in the free wall and the septal wall (Table V). Marked myocardial fiber disorganization (grade 2+ or 3+) were predominantly seen in the junctional area between the left ventricular free wall and the ventricular septum. In the ventricular septum near the junctional area, a large focus of marked fiber disorganization (relatively broad bundles of muscle cells were oriented at oblique or perpendicular angles to each other) was more frequently observed in hypertrophied hearts of the newborn from the mother rats treated with isoproterenol than in the control (Table VI and Fig. 3-C, D). By an electron microscope, the vast majority of myofibrils were normally arranged (Fig. 4-A), while disorganized myofibrils were often found in hypertrophied, abnormally arranged cardiac muscle cells of isoproterenol-

treated newborn rats (Fig. 4-B, C). However, myofibrillar disarray was also frequently observed in the cardiac muscle cells of the junctional area and occasionally in the ventricular septum or left ventricular free wall in the control newborn especially near the intercalated discs (Fig. 4-D).

Rats of 4 and 7 weeks of age

Body weight, ventricular weight and ventricular weight to body weight ratio are summarized in Table III. Significant ventricular hypertrophy still remained in the offspring of the mother rats treated with isoproterenol. However, the disproportionate septal hypertrophy of the isoproterenol-treated offspring became obscure at the 4th week (Table IV), and disappeared at the 7th week (Table IV and Fig. 5). The mean

fiber diameter of isoproterenol-treated animals was significantly larger at the 4th weeks in both the free wall and the septal wall, which became equal to that of the control at 7 weeks of age (Table V). In each of the control and isoproterenol-treated rats at 4 and 7 weeks of age, the vast majority of cardiac muscle cells and myofibrils in the ventricular septum were normally arranged (i.e. were in parallel alignment) (Table VI and Fig. 6-A, B).

DISCUSSION

The present study shows that the administration of isoproterenol during the fetal period can produce cardiac hypertrophy, disproportionate septal hypertrophy, intra- and intercellular disarray in rats. These morphological changes are similar to asymmetric septal hypertrophy with disorganized cardiac muscle cell, the most characteristic anatomic feature of hearts with hypertrophic cardiomyopathy.¹¹⁻¹⁷ Olsen⁶⁻⁸ reported that cardiac hypertrophy and intra-cellular disarray could be produced by exposure of rats to triac during fetal development. However, hypertrophic cardiomyopathy is characterized by often extreme cardiac hypertrophy, asymmetric septal hypertrophy, intra- and intercellular disarray. The experiment described in this paper confirms all of these abnormal changes.

Olsen observed the morphological changes in the hearts of the offspring from the mother rats treated with triac at 2 and 7 days of age, while those anatomical features observed in patients with hypertrophic cardiomyopathy usually manifest themselves clinically in adults. Therefore, we investigated the effects produced by the exposure of rats to isoproterenol during fetal development at 2 days, and 4 and 7 weeks after birth. The morphological changes observed in the hearts of the isoproterenol-treated newborns became obscure at the 4th week and progressively disappeared at the 7th week. Maron et al reported that disproportionate thickening of the ventricular septum was a common finding in the developing hearts of normal human embryos and during subsequent pre- and postnatal growth, disproportionate septal thickening became progressively less common.¹⁸ It is probably more likely that the development of disproportionate septal hypertrophy in patients with hypertrophic cardiomyopathy occurs by mechanisms that interfere with normal cardiac development.

The cause of hypertrophic cardiomyopathy is unknown, but a number of clinical clues suggest a link with catecholamine function.^{2,19,20} One hypothesis is that the timing and extent of cardiac adrenergic innervation is critical such that increased or temporally inappropriate secretion of nerve growth factor leading to excessive adrenergic stimulation (to which the fetal heart is known to be sensitive) could lead to myofibrillar disarray and failure of normal regression of the ratio between septal and free wall thickness; this mechanism could lead to the development of hypertrophic cardiomyopathy. However, we observed that a myofibrillar disarray was a common finding in the hearts of the normal newborn rats and the disorganized myofibrils in the hypertrophied, abnormally arranged cardiac muscle cells of isoproterenol-treated newborn rats spontaneously disappeared during subsequent postnatal growth and disproportionate septal hypertrophy disappeared.

In the present study, we succeeded in producing the morphological changes in the newborn offspring of isoproterenol-treated pregnant rats similar to those found in patients with hypertrophic cardiomyopathy. This lead us to believe that abnormal catecholamine function in the fetal period may result in disproportionate hypertrophy of the ventricular septum. However, these morphological changes observed in the newborn heart progressively disappeared during postnatal growth. Recent observations on blood noradrenaline levels in patients with hypertrophic cardiomyopathy suggest that catecholamine responses are abnormal.²¹ In addition to the abnormal catecholamine function during the fetal period, subsequent postnatal abnormality of interaction between the adrenergic stimulus and myocardial adrenergic receptor sites may be also important in initiating disproportionate septal hypertrophy.

REFERENCES

1. ELLIOTT CM, et al: Idiopathic hypertrophic sub-aortic stenosis associated with cutaneous neurofibromatosis: report of a case. *Am Heart J* 92: 368, 1976
2. GOODWIN JF: Prospects and predictions for cardiomyopathies. *Circulation* 50: 210, 1974
3. POLANI PE, MOYNIHAN EJ: Progressive cardiomyopathic lentiginosis. *QJ Med* 162: 205, 1972
4. SOMERVILLE J, BONHAM CARTER RE: The heart in lentiginosis. *Br Heart J* 34: 58, 1972
5. PERLOFF JK: Pathogenesis of hypertrophic cardiomyopathy: hypotheses and speculations. *Am Heart J* 101: 219, 1981

6. OLSEN EGJ, SYMONS C, HAWKEY CM: Effect of triac on the developing heart. *Lancet* 2: 221, 1977
7. PEARCE PC, HAWKEY CM, SYMONS C, OLSEN EGJ: Effect of triac and β -adrenergic blocking agents on the myocardium of developing rats. *Cardiovasc Res* 17: 7, 1983
8. PEARCE PC, HAWKEY C, SYMONS C, OLSEN EGJ: Role of calcium in the induction of cardiac hypertrophy and myofibrillar disarray. *Br Heart J* 54: 420, 1985
9. ROBKin M, SHEPARD TH, BAUM D: Autonomic drug effects on the heart rate of early rat embryos. *Teratology* 9: 35, 1977
10. KAWAMURA K, KASHII C, IMAMURA K: Ultrastructural changes in hypertrophied myocardium of spontaneously hypertensive rats. *Jpn Circ J* 40: 1119, 1976
11. CLARK CE, HENRY WL, EPSTEIN SE: Familial prevalence and genetic transmission of idiopathic hypertrophic subaortic stenosis. *N Engl J Med* 289: 709, 1973
12. van DORP WG, ten CATE FJ, VLETTER WB, DOHMEN H, ROELANDT J: Familial prevalence of asymmetric septal hypertrophy. *Eur J Cardiol* 4/3: 349, 1976
13. EPSTEIN SE, HENRY WL, CLARK CE, ROBERTS WC, MARON BJ, FERRANS VJ, REDWOOD DR, MORROW AG: Asymmetric septal hypertrophy. *Ann Intern Med* 81: 650, 1974
14. ABBASI AS, MACALPIN RN, EBER LM, PEARCE ML: Left ventricular hypertrophy diagnosed by echocardiography. *N Engl J Med* 289: 118, 1973
15. MENGES H, BRANDENBURG RO, BROWN AL: The clinical, hemodynamic and pathologic diagnosis of muscular subvalvular aortic stenosis. *Circulation* 24: 1126, 1961
16. ROBERTS WC: Valvular, subvalvular and supra-valvular aortic stenosis: Morphologic features. *Cardiovasc Clin* 5: 104, 1973
17. ABBASI AS, MACALPIN RN, EBER LM, PEARCE ML: Echocardiographic diagnosis of idiopathic hypertrophic cardiomyopathy without outflow obstruction. *Circulation* 46: 897, 1972
18. MARON BJ, VERTER J, KAPUR S: Disproportionate ventricular septal thickening in the developing normal human heart. *Circulation* 57: 520, 1978
19. GOODWIN JF: An appreciation of hypertrophic cardiomyopathy. *Am J Med* 68: 797, 1980
20. GOODWIN JF: Hypertrophic cardiomyopathy: a disease in search of its own identity. *Am J Cardiol* 45: 177, 1980
21. GOODWIN JF: Cardiomyopathy: an interface between fundamental and clinical cardiology. In *Proceedings of the VIII World Congress of Cardiology*, ed by HAYASE S, MURAO S, Elsevier-North-Holland, Tokyo. Amsterdam, 1979, p 103