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## Cardiac hypertrophy in surgically denervated dogs with aortic stenosis

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*Summary.* Left ventricular cell hypertrophy in dogs with aortic stenosis was accelerated by surgical denervation of the left ventricle. We conclude that there are neural mechanisms which, when present, inhibit cardiac cell hypertrophy.

We have reported previously that cardiac cell hypertrophy is accelerated by left cervical sympathetic denervation in spontaneously hypertensive rats[2]. In that study, however, the possibility existed that a genetic factor could have participated in the acceleration of the cardiac cell hypertrophy. The present study was designed to exclude the genetic factor, and to employ the pressure factor alone in the production of cardiac cell hypertrophy.

*Materials and methods.* 18 adult mongrel dogs (12–17 kg) were anesthetized by sodium pentobarbital (30 mg/kg), and artificially ventilated. A left thoracotomy was performed through the 4th intercostal space. The ascending aorta was carefully separated from the main pulmonary artery at a distance 1 cm from the aortic valve and stenosed with a polyethylene band 4 mm wide, monitoring pressure by a catheter (NIH; 7F). The pressure gradient across the aortic valve after aortic stenosis ranged from 30 to 55 mm Hg.

After the operation of aortic stenosis, 10 of the dogs underwent surgical denervation of the left ventricle, using the technique of Geis et al.[3]. The remaining group was sham operated. Surgical denervation of the left ventricle was confirmed by the absence of elevation of the left

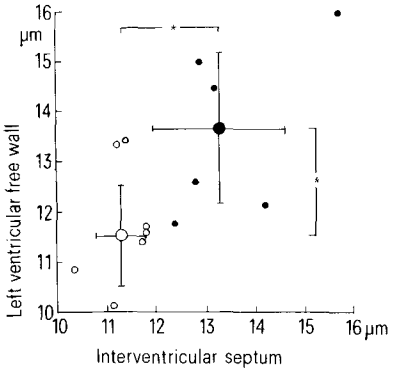


Figure 1. Cell diameters of the left ventricular free wall and the interventricular septum in the denervated group (●) and the sham-operated group (○), which are measured in cross sections at the level of the nuclei. The cell diameters in the denervated group are significantly larger than those in the sham-operated group. Each small circle indicates the mean diameter of 50–60 cells in each dog. Large circles show the mean values for 7 denervated dogs (●) and 6 sham-operated dogs (○). Mean ± SD are shown. * $p < 0.05$.

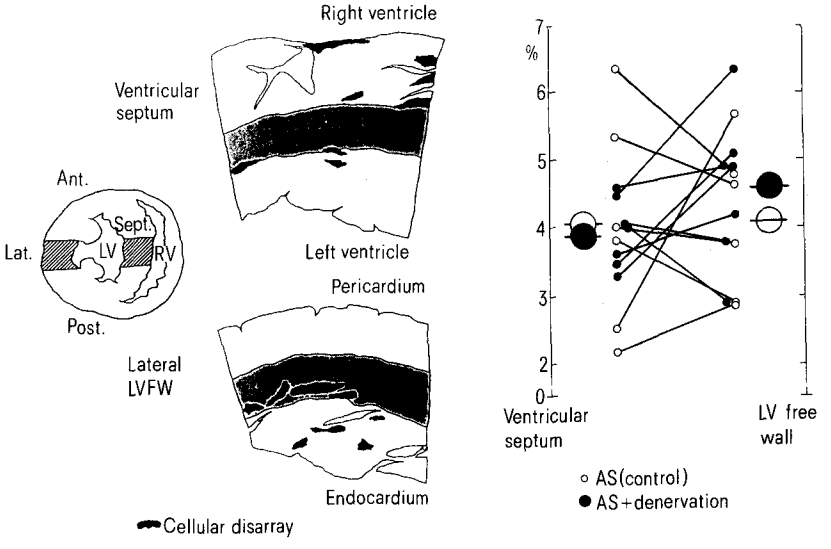


Figure 2. The areas of cellular disorganization in the interventricular septum and the left ventricular free wall measured by the method of Aron et al.[4]. The left figures show a scheme representing the location measured by the method of Aron and his collaborators, and the areas of the cellular disorganizations. The areas in the left ventricular free wall in the denervated group are larger than those in the sham-operated group. However, there are no statistically significant differences between the two.

ventricular pressure due to left stellate ganglion stimulation (5 V; 1 msec; 10 sec in duration). 5 dogs died within the 1st week after operation, despite the use of postoperative antibiotic therapy. Finally, 6 of the sham-operated group and 7 of the denervated group were alive 4 weeks after operation. Electrocardiographic examination was done every week. The analyses were performed according to the criteria for left ventricular hypertrophy.

4 weeks after operation, the pressure gradients across the aortic valve of both groups were measured again. The mean values of the sham-operated and the denervated groups were $34.1 \pm 13.9$ mm Hg (mean $\pm$ SD) and $20.0 \pm 7.0$ mm Hg, respectively. No significant difference was found between the two. The heart was removed under sodium pentobarbital anesthesia. The tissue was immediately fixed with 10% formalin for light microscopic examination. The significance of the means was calculated by Student's paired or unpaired t-test, as appropriate.

*Results and discussion.* 4 weeks after denervation, the electrocardiogram was markedly changed: The algebraic sum of $R_I + R_{III}$ in the denervated group was significantly increased ($p < 0.05$). The summations of $S_{VI} + R_{V5}$ and $R_{V5} + S_{aVF} + S_{VI}$ were also increased in the denervated group, whereas the sham-operated group showed a statistically significant decrease in the summations ($p < 0.005$). The summation of the amplitudes of T waves in the precordial leads ($V_{1\text{-}5}$) in the denervated group was significantly increased from $19.3 \pm 9.5$ mm to $39.8 \pm 12.5$ mm ($p < 0.001$). In contrast, the sham-operated group showed decreased amplitudes. The sum of the amplitudes may decrease because of the influence of the thoracotomy. The thoracotomy might produce the pericardial or pleural effusion or adhesion, leading to an enhancement of electrical resistances. On the other hand, the sum of the amplitude in the denervated group results from cardiac cell hypertrophy. The influence of the thoracotomy on the amplitudes may be reduced by the effect of cardiac cell hypertrophy.

Histologically, the cell diameters in the free wall of the left ventricle and in the interventricular septum were measured in cross sections at the level of the nuclei. The cell diameters of the left ventricular free wall and the interventricular septum in the denervated group were $13.62 \pm 1.58$ µm (mean $\pm$ SD) and $13.22 \pm 1.37$ µm, respectively. Those in the sham-operated group were $11.48 \pm 1.07$ µm and $11.31 \pm 0.58$ µm, respectively. Statistically significant differences were found between those cell diameters in both groups ($p < 0.05$; fig. 1). The areas of cellular disorganization in the free wall of the left ventricle and the interventricular septum were also measured by the method of Aron et al.[4]. The areas on the left ventricular free wall in the denervated group were larger than in the sham-operated group: the one was $4.604 \pm 1.087\%$ (mean $\pm$ SD) and the other was $4.098 \pm 1.147\%$ (fig. 2). No significant difference was found between the two. In the interventricular septum, however, the areas remained unchanged in both groups. The ratio of heart wt to body wt in the denervated group was greater than that in the sham-operated group: the one was $0.962 \pm 0.138\%$ (mean $\pm$ SD) and the other was $0.929 \pm 0.030\%$, having no significant differences. The body wt did not change during the experimental period. Thus, electrocardiographic measurements and cell diameters in the denervated group were significantly greater than those in the sham-operated group. These findings may provide evidence of cardiac hypertrophy in the denervated dogs.

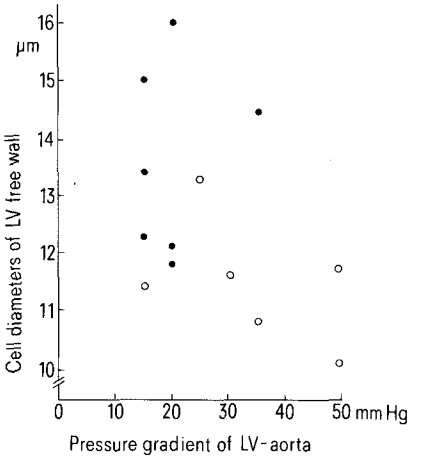


Figure 3. Lack of correlation between the pressure gradient across the aortic valve 4 weeks after the aortic stenosis and the cell diameters of the left ventricular free wall. Circle show the mean cell diameter of 50–60 cells in each denervated dog (●) and each sham-operated dog (○).

In the study, one of the major factors influencing cardiac cell hypertrophy would be the pressure-overload induced by aortic stenosis. However, no significant correlations were found between the pressure gradient across the aortic valve and cell diameters of the left ventricular free wall 4 weeks

after operation (fig. 3). The cell diameters in the denervated dogs became large as compared to those in the sham-operated group, even though the pressure gradient was small.

The results presented in this paper, together with the previous study[3], suggest that cardiac cell hypertrophy is accelerated by denervation of the heart independently of the degree of pressure-overload or genetic factor. This may imply a neural mechanism which, when present, would inhibit cardiac cell hypertrophy. The mechanism by which cardiac cell hypertrophy is increased after denervation is not clear. However, a report says that denervation leads to a supersensitivity to chemical and physical stimuli[5], and also results in an increase in the number of adrenergic receptors[6]. Left stellate ganglectomy may lead to a reduction in free lysosomal enzyme activity, implying a possible reduction in protein degradation in the cell[7]. Furthermore, inhibition of glycolysis has been observed in the denervated dog heart[8]. In conclusion, our data supply evidence that cardiac cell hypertrophy in dogs with aortic stenosis may be accelerated by denervation of the heart.

1 This work was supported by a grant of the Ministry of Education in Japan for 1981.

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## Granules of Langerhans cells in the thymus contain gold

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*Summary.* The granules of Langerhans cells in the thymus of the rat were subjected to X-ray microanalysis. It was established that gold is present in these morphological structures.

Langerhans cells contain characteristic granules (fig. 1), the ultrastructure of which was described for the first time by Birbeck and Breathnach[1]. Despite numerous studies, their role is still not clear[2-5], so any new information about the granules is of potential significance. With the above in mind we attempted to examine the chemical content of Langerhans cell granules using X-ray analysis.

*Material and methods.* Studies were performed on male Wistar rats aged 60 days and fed standard laboratory animal chow LSM (Laboratory Diet Factory, Motycz, Poland). The animals were decapitated and small thymus fragments were fixed for 1 h in 2.5% glutaraldehyde in 50 mM phosphate buffer, pH 7.4. The material was post-fixed in 1% $OsO_4$ solution in 0.1 M phosphate buffer, pH 7.4, dehydrated in a series of alcohols, passed through acetone and embedded in a low viscosity medium (Spurr, 1969). 100-nm thick sections were collected on nylon grids and subjected to X-ray microanalysis. The analysis was performed using a Link EDX 290 analyzer coupled to a Zeiss EM 10 electron microscope. The emission spectra

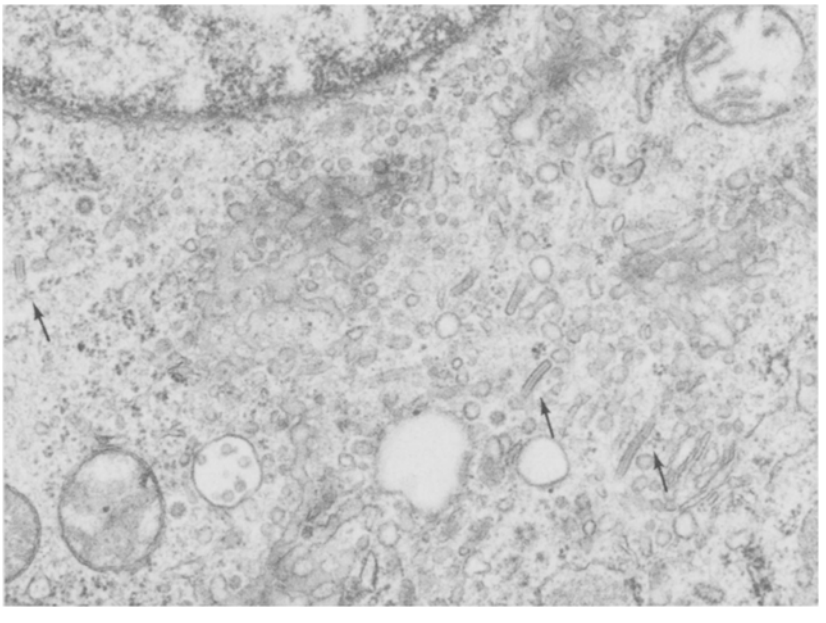

Figure 1. Langerhans cell of rat thymus. Arrows indicate some of the Langerhans cell granules. ×26,000.