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POST-NATAL DEVELOPMENTAL CHANGES IN THE LENGTH–TENSION RELATIONSHIP OF CAT PAPILLARY MUSCLES

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SUMMARY

1. The length–tension relationship and the effect of paired electrical stimulation were studied in right ventricular papillary muscles obtained from cats. The animals were divided into three age groups: neonatal kittens, less than 24 hr of age; infant kittens, 16–18 days of age; and adult cats.

2. The muscles were electrically driven to contract isometrically using field stimulation at 10–20% above the threshold value.

3. At $L_{\max}$ the neonatal preparations produced significantly less developed (active) tension than did the adult preparations. The resting tension at $L_{\max}$ was significantly greater in the neonatal preparations than in the adult ones. The infant group occupied an intermediate position between the two other groups.

4. The response to paired electrical stimulation was less in the neonatal group than in either the infants or the adults.

5. It is concluded that post-natal developmental changes occur in the contractile properties of the myocardium of the cat.

INTRODUCTION

In the mammal, the change from placental to lung gas exchange which occurs at birth causes profound changes in the route of the circulation and the structure of the cardiovascular system (Dawes, 1968). As a consequence of these changes longer term alterations in the relative muscle masses of the right and left ventricles ensue (Hort, 1966; Keen, 1955), the right ventricle becoming relatively less massive when compared to the left ventricle. This change in relative muscle mass appears to be associated with changes in the relative compliance of the two ventricles, the right ventricle becoming more compliant than the left ventricle (Romero, Covell & Friedman, 1972). However, both ventricles show an absolute increase in compliance with age. To some extent this increasing compliance could be explained by the Laplace relationship since both ventricles are increasing in cavity size. However, the increasing absolute compliance of the ventricles raises the question of whether this is in part due to developmental changes in the properties of the myocardium, for example in the length–tension relationship. In this study we have examined the length–tension relationship and the effect of paired stimulation in cat right ventricular papillary muscles during the period after birth and compared these findings to those seen in the adult cat.

METHODS

Material. The animals were divided into three groups which will be described as neonates (kittens under 24 hr of age), infants (kittens 16–18 days of age) and adults (mature cats, less than 2 kg body wt.). In all cases right ventricular papillary muscles were used. The length–tension relationship was examined in detail in six neonates, eight infants and eight adult cats. The effect of paired stimulation was examined in these muscles and in an additional six neonates, nine infants and one adult.

Procedure. The neonates and infants were killed with a blow to the neck, the adults were anaesthetized with intraperitoneal Nembutal (30 mg/kg). In all animals the hearts were rapidly removed and dissected in cooled physiological electrolyte solution (NaCl, 7·306 g/l.; KCl, 0·32 g/l.; $MgCl_26H_2O$, 0·203 g/l.; $NaH_2PO_42H_2O$, 0·156 g/l.; $CaCl_22H_2O$, 0·367 g/l.; $NaHCO_3$, 2·1 g/l. and D-glucose 2 g/l.) bubbled with 95 % oxygen and 5 % CO_2. The muscles were mounted vertically in a water-jacketed Perspex bath, the lower ends of the muscle being rigidly fixed and the tendinous end being attached to a force transducer (Statham UC3 with microscale attachment) via a fine rigid straw. The chamber was filled with the solution described above which was maintained at 30° C and was constantly bubbled with 95 % oxygen and 5 % CO_2. This maintained the pH at 7·4 and the P_{O_2} in excess of 300 mmHg.

The preparations were driven to contract isometrically using field stimulation via platinum electrodes. Stimulation was provided by a constant voltage square wave generator set at 10–20 % above the threshold for the muscle. Unless otherwise stated the frequency of stimulation was 0·4 Hz.

The muscle chamber was rigidly mounted on a myograph stand with the force transducer mounted on the moving platform of the stand. The stand was fixed to a steel anti-vibration base. Increments in length of the muscle were obtained by elevating the moving platform. These increments were monitored using a micrometer dial gauge. Resting and developed force were recorded on a Devices MX4 direct writing recorder.

All preparations were allowed to stabilize for 30 min before the experiments were started. Length–tension curves were obtained by increasing the length of the muscle in increments of 0·01–0·02 mm until the length at which maximum developed (active) force was noted (L_{max}) had just been exceeded. The muscle was returned to the length at which developed force was just detectable and the procedure was repeated. On the second run the resting and developed force were measured 2 min after each increment in length.

The muscle was then returned to L_{max} and the effect of paired stimulation was tested. Paired pulses were delivered, the pairing intervals being increased from 300 to 800 msec in 100 msec increments. The maximum increase in developed force was taken as the response to paired stimulation. This procedure was carried out on all the muscles. However, when the data were analysed it was possible to construct complete length–tension curves (see below) in six neonates, eight infants and eight adults. In the remainder, one or more points on the ascending limb of the curve could not be calculated. Data from these muscles were therefore only used in the assessment of the effect of paired electrical stimulation.

At the close of the experiment, muscle length was measured *in situ* using a Vernier microscope, the muscle was then removed from the chamber, cut free from its attachments, blotted dry and weighed in an airtight weighing bottle on a five-place balance. From the length and weight and assuming a specific gravity 1, the cross-sectional area of each muscle was determined; in all cases this was less than 0·7 mm². Force was then expressed as stress (force/mm²).

Resting and developed stress were calculated at intervals of 5 % L_{max} over the range 75–105 % L_{max}. In each of the three age groups the stress data at each length were pooled and the length–tension curve for that age group was plotted with the mean values thus obtained. Linearity of the ascending limb of the length–tension curve was assessed using bivariate linear regression analysis of the mean values. The control value for the response to paired electrical stimulation was taken as the developed force before the start of paired stimulation. The response was expressed as % increase over this control value. Comparison between age groups was made using Student's t test with appropriate corrections for small samples of unequal size and unequal variance. The null hypothesis was retained when its probability, P, exceeded 0·05.

RESULTS

Light–tension curve

Adult cats. There were papillary muscles from eight animals in this group. The relationships of resting and developed stress to length are shown in Fig. 1. In the case of developed stress this relationship was linear over the range 75–100 % L_{max}, correlation coefficient (r) = 0·985; testing for significance of the slope gave t = 13·828 ($P < 0·001$). Developed stress at L_{max} was 2·30 g/mm² ± 0·34 (S.E. of mean) and resting stress at L_{max} was 1·17 g/mm² ± 0·26 (S.E. of mean).

Infant kittens. There were papillary muscles from eight animals in this group. The relationships of resting and developed stress to length are shown in Fig. 2. As in adult cats the relationship of developed stress to length over the range 75–100% L_{max} was linear, correlation coefficient (r) = 0·995, testing for significance of the slope gave t = 24·392 ($P < 0·001$). Developed stress at L_{max} was 1·65 g/mm² ± 0·32 (S.E. of mean) and resting stress at L_{max} was 1·94 g/mm² ± 0·27 (S.E. of mean).

Neonatal kittens. There were papillary muscles from six animals in this group. The relationship of resting and developed stress to length are shown in Fig. 3. As in the preceding age groups this relationship was linear in the case of developed stress, correlation coefficient (r) = 0·981, testing for significance of the slope gave t = 9·797 ($P < 0·001$). Developed stress at

L_{max} was 0·98 g/mm^2 ± 0·37 (S.E. of mean) and resting stress at L_{max} was 2·37 g/mm^2 ± 0·40 (S.E. of mean).

Statistical comparisons showed that at 1-L_{max} neonatal papillary muscle developed significantly less active tension than did adult preparations

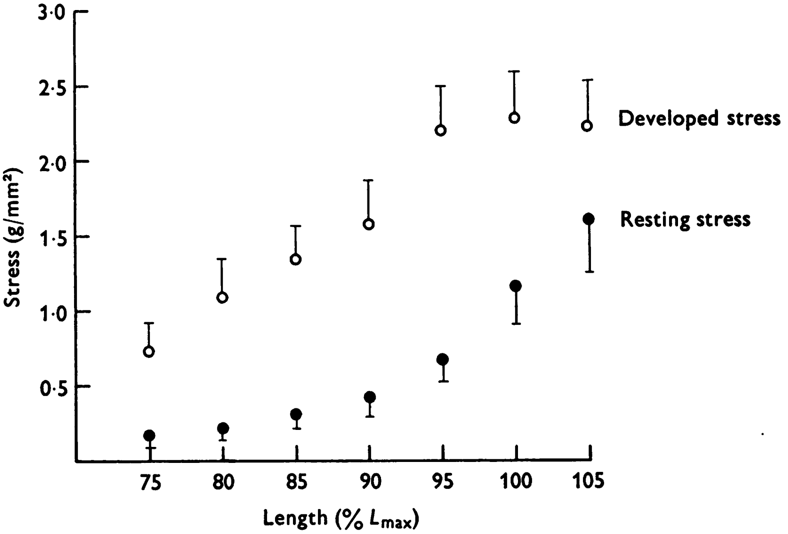

Fig. 1. Length–tension curve obtained in right ventricular papillary muscles from eight adult cats. Circles represent means, the bars equal ± 1 S.E. of mean.

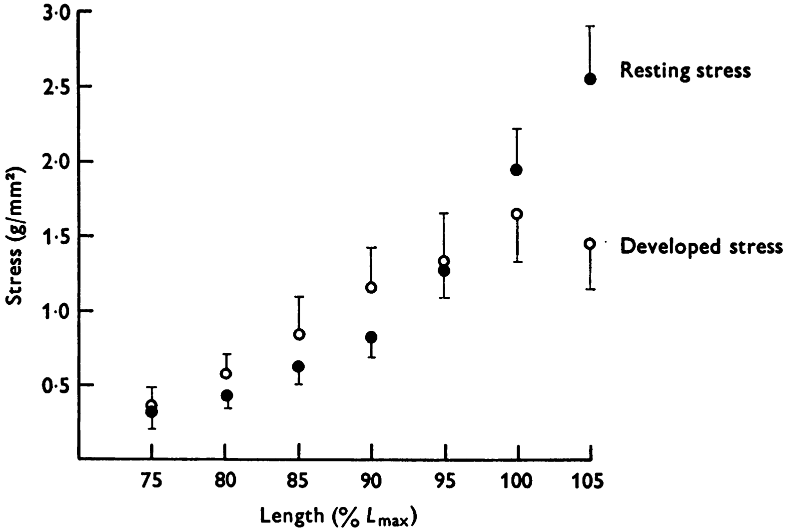

Fig. 2. Length–tension curve obtained in right ventricular papillary muscles from eight infant kittens, aged 16–18 days. Circles represent means, the bars equal ± 1 S.E. of mean.

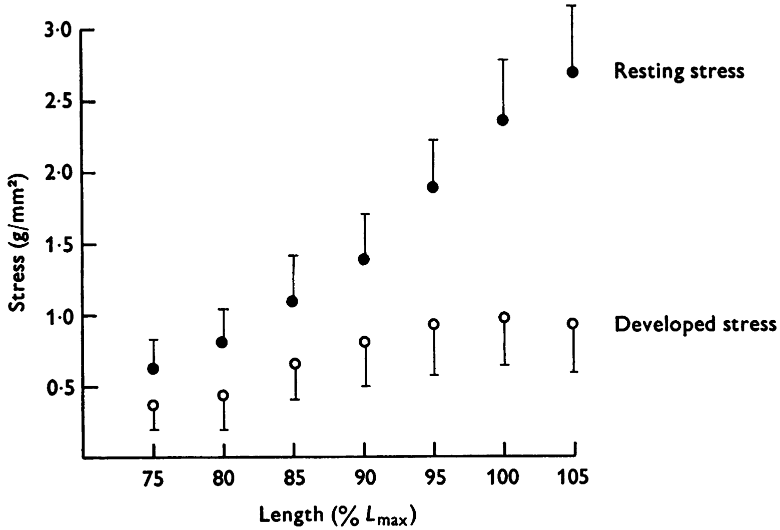

Fig. 3. Length–tension curve obtained in right ventricular papillary muscles from six neonates under 24 hr of age. Circles represent means, the bars equal ± 1 S.E. of mean.

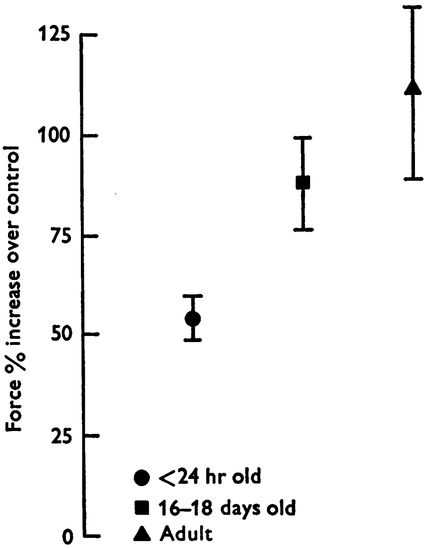

Fig. 4. Maximum effect of paired electrical stimulation. Symbols represent means, the bars equal ± 1 S.E. of mean.

($P < 0{\cdot}02$) and that the resting tension was significantly greater in the neonatal muscles ($P < 0{\cdot}05$). Infant muscles appeared also to develop less active tension than adult preparations but significance was not proven ($P = 0{\cdot}05$). No other statistical significant differences were found.

Effect of paired electrical stimulation (*Fig. 4*)

Adult cats. There were nine papillary muscles in this group. The maximum % increase in developed force was 101 % ± 12 (S.E. of mean).

Infant kittens. There were seventeen papillary muscles in this group. The maximum % increase over control was 88% ± 12 (S.E. of mean).

Neonatal kittens. There were twelve papillary muscles in this group. The maximum increase over control was 53% ± 6 (S.E. of mean).

Statistical analysis revealed significantly smaller responses in the neonatal muscles when compared with the infant group ($P < 0{\cdot}01$) and the adult group ($P < 0{\cdot}05$). However, statistically significant difference was not shown between the infant and adult groups.

DISCUSSION

The results presented in this paper demonstrate that the length–tension relationship of neonatal kitten papillary muscle is significantly different from that of the adult. The neonatal papillary muscle produced less developed (active) tension than the adult at a significantly higher resting tension. The infant group occupied an intermediate position between the neonates and the adults. The response to paired electrical stimulation was also smaller in neonatal papillary muscles than in either the infant or adult preparations.

It is unlikely that these differences were related to the methods used, since apart from the method of killing the animals, the same procedure was used in all three groups. The lower developed tensions seen in preparations from younger animals are unlikely to be due to hypoxia because adequate oxygenation was provided and all papillary muscles had a cross-sectional area of less than 1 mm^2, thus being well within the maximum size suggested by Parmley & Sonnenblick (1971). In fact the infant muscles were all less than 0·5 mm^2 in cross-sectional area and thus are also within the size limit suggested by Cranefield & Greenspan (1960). Furthermore the fact that in all three groups the ascending limb of the length–developed tension curve was approximately linear (Sonnenblick, Spiro & Cottrell, 1963), suggests that these results are not artifacts but represent real age-related differences in myocardial function.

There have been two previous reports of age-related differences in the responses of isolated myocardial preparations. In the first of these (Boerth, 1972) the cat papillary muscle was used. The length–developed tension curves and the response to paired electrical stimulation were qualitatively similar to those in the present paper. There were quantitative differences. For example the maximum developed stress in the group with a mean age of one day was 0·3 g/mm^2 ± 0·1 (S.E. of mean), which is only 30 % of the maximum stress in our neonatal group. Unfortunately Boerth (1972) gives no data concerning resting stress seen in his preparations. In the second series (Friedman, 1972) right ventricular moderator bands from foetal and adult sheep were used. No information is given about the size of the preparations employed, thus in an animal as large as the sheep, the data must be treated with caution. However, the length–developed tension curve and the length–resting tension curve appear to show qualitatively similar changes with age to those in the present report. No stronger statement can be made because no numerical data and no statistical analysis were included.

The inability of neonatal myocardium to generate adult levels of force when contracting isometrically would account for the deterioration of left ventricular function in the new-born lamb at aortic pressures which are normal for the adult (Downing, Talner & Gardner, 1965). The high resting tension found in the new-born at $L_{\max}$ supports the hypothesis that the fall in absolute compliance of the left ventricle which occurs with age (Romero *et al.* 1972) has a structural basis.

Our expiermental data together with the available information in the literature strongly suggest that the myocardium matures during late foetal and early post-natal life. It is possible that the lower developed tensions seen in the immature cat myocardium may be related to the effect of field stimulation on catecholamine release (Jewell & Blinks, 1968). Noradrenaline might be released by the adult myocardium but not by the neonate, because in many species including the cat, myocardial sympathetic innervation may not be complete at birth (Glowinski, Axelrod, Kopin & Wurtman, 1964; Friedman, Pool, Jacobowitz, Seagren & Braunwald, 1968; Lebowitz, Novick & Rudolph, 1972; and Patricia Davies & M. Tynan, unpublished). The use of field stimulation would not explain the high resting tensions seen in the immature preparations and the voltages used should have kept catecholamine release to a minimum. Therefore it would appear that other explanations are needed.

In skeletal muscle from immature pigs and humans, less contractile protein is present than in the adult (Dickerson & Widdowson, 1960). In the same species, immature myocardium contains less protein nitrogen than the adult (Widdowson & Dickerson, 1960). If the rise in myocardial protein nitrogen reflects an increase in contractile protein this could explain the age-related increase in contractile force but would not explain the decrease in myocardial stiffness which occurs with maturation. It is tempting to attribute the greater stiffness of immature heart muscle to a higher connective tissue content. This would not appear to be the case because hydroxyproline levels in the sheep increase with age (Romero *et al.* 1972). Friedman (1972) suggests that immature heart muscle contains a greater proportion of non-contractile protein such as surface membranes and that this would explain the increased stiffness in both isolated myocardial preparations and in the intact hearts from immature animals.

Age-related changes in myocardial performance undoubtedly exist and knowledge of them is necessary for the interpretation of physiological and pharmacological experiments. However, the explanation of these changes in still speculative.

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