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Decrease in fibre numbers of dog pectineus muscle with age

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INTRODUCTION

The maximum number of muscle fibres in a muscle is generally fixed before birth or shortly after (Goldspink, 1972). Thereafter it is widely assumed that the number of muscle fibres remains constant throughout the life of the animal. This conclusion is based on the results of workers who have studied fairly young animals. However, some workers who have studied the muscles of older animals have reported that the number of muscle fibres in a given muscle does not remain unchanged throughout life, but in fact decreases with age.

In a mixed muscle like the pectineus of the dog there are three types of muscle fibre which can be characterized histochemically as: Type I (slow contracting), Type II (fast contracting), and intermediate. Decrease in the total number of muscle fibres with age could result from selective loss of one or two particular muscle fibre types, or it could result from loss of some of all three types of fibre. The purpose of this study was first to determine whether or not there is a decrease in the total number of muscle fibres in the pectineus muscle of the dog with age and if so to determine whether the decrease is due to loss of one or two particular muscle fibre types or to the loss of fibres in all three groups.

MATERIALS AND METHODS

Eighteen normal dogs (7 males, 11 females) were studied. Unilateral myectomies of pectineus muscles were performed when the dogs were 2 months old. Right myectomies were performed on 10 dogs (3 males and 7 females) and left myectomies on 8 dogs (4 males and 4 females). The contralateral muscles were removed when the dogs were 12 months old. The side chosen for the initial myectomy was determined by a random sampling procedure. In removing the muscles, general anaesthesia was induced by and maintained with intravenous sodium thiamylal. The dogs were positioned in dorsal recumbency and the hind limbs were gently secured in an abducted position. The pectineus muscles were then removed aseptically.

Gross morphology of the pectineus muscle

The pectineus muscle in the dog is fusiform. It arises tendinously from the prepubic tendon and is inserted tendinously on the caudal surface of the femur distal to its middle. Its fibres converge on the tendons at each end. Each fibre extends from the tendon of origin to the tendon of insertion. The thickest part of the muscle belly is in its proximal part. Transverse sections were therefore cut here in the expectation that they would include sections of all the fibres present in the muscle.

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Histochemical procedures

The sections were frozen in 2 methyl-butane cooled to − 125 °C in liquid nitrogen. Cryostat sections of 10 μm thickness were then cut. The sections from both the young and older muscles were incubated for the same length of time for the histochemical demonstration of myofibrillar ATPase according to the method of Padykula & Herman (1955) as described by Cardinet (1971). This method applied to the canine pectineus muscle differentiated three fibre types: Type I, or slow contracting (light staining), Type II or fast contracting (dark staining) and intermediate (staining intermediate between Type I and Type II fibres). When other enzymic reactions were employed, Type II fibres were characterized by high phosphorylase and low NAD-diaphorase reactions, while Type I fibres were characterized by a reciprocal profile for those enzymes.

Determination of numbers of muscle fibres

The number of fibres contained in each section at 2 months and 12 months of age respectively was determined by the indirect muscle fibre counting method of Jimnez, Cardinet, Smith & Fedde (1975). The histochemical types were distinguished while counting the muscle fibres, so that the number of Type I, Type II and intermediate fibres, as well as the total number of fibres, could be estimated.

Statistical analyses

The results obtained at 2 months and 12 months of age were statistically analyzed with means and standard errors being calculated. Analysis of variances (Student's '*t*' test) was also used to examine whether differences between the numbers of muscle fibres at 2 months and 12 months of age were statistically significant (Snedecor, 1965; Fisher & Yates, 1967). The analyses were made according to sex.

RESULTS

The total number of muscle fibres per muscle was smaller at 12 months than at 2 months in all the muscles studied, the decrease being 31·8 % in males and 32·5 % in females. The difference was highly significant in both male ($P < 0{\cdot}01$) and female ($P < 0{\cdot}001$) as may be observed from Table 1. The numbers of Type I muscle fibres were also significantly smaller in the 12 months as compared with the 2 months old dogs in both males ($P < 0{\cdot}02$) and females ($P < 0{\cdot}05$) (Table 1), the decrease being 22·5 % in males and 18·0 % in females. The number of Type II muscle fibres also decreased significantly between 2 and 12 months in males ($P < 0{\cdot}01$) and females ($P < 0{\cdot}01$), the decrease amounting to 37·4 % in males and 41·0 % in females (Table 1). Between 2 and 12 months of age the number of intermediate muscle fibres decreased by 77·4 % in the males and by 69·3 % in the females. These differences were highly significant for both male ($P < 0{\cdot}01$) and female ($P < 0{\cdot}01$) dogs (Table 1). No significant differences were observed between the sexes or between left and right sides.

Table 1. *Numbers of muscle fibres per pectineus muscle (mean ± S.E. given for each measurement)*

Sex	Fibre types	No. of fibres at 2 months of age	No. of fibres at 12 months of age	Student's 't' test *P*	Degrees of freedom
Males	All	149827 ± 10205	102218 ± 7700	< 0·01	12
	Type I	63783 ± 4902	49427 ± 3882	< 0·02	12
	Type II	84099 ± 7221	52668 ± 4936	< 0·01	12
	Intermediate	1945 ± 306	439 ± 20	< 0·01	12
Females	All	148026 ± 6218	99939 ± 5513	< 0·001	20
	Type I	58324 ± 3472	47847 ± 2921	< 0·05	20
	Type II	86984 ± 3410	51257 ± 2423	< 0·01	20
	Intermediate	2718 ± 445	835 ± 376	< 0·01	20

DISCUSSION

It was clear from this study that the total number of muscle fibres, as well as the numbers of the different types of muscle fibre, in both male and female dogs, decreased very significantly between 2 and 12 months of age. This decrease amounted to a loss of about 32 % of the total number of muscle fibres present in the muscles of both sexes at 2 months of age. The decrease resulted from the loss of about 20 % of the Type I, 39 % of the Type II and 73 % of the intermediate muscle fibres present when the dogs were 2 months old. Decrease in the total number of muscle fibres has been reported also in man (Montgomery, 1962), pig (Staun, 1963), cattle (Bendal & Voyle, 1967) and rat (Rayne & Crawford, 1975). This is contrary to Rowe & Goldspink (1969), who stated that the total number of muscle fibres remained statistically unchanged throughout the life of an animal. They studied mice from 1 day after birth to 6 months of age. In the case of man decrease was observed between 13 months and 64 years of age. In the pig, even though the exact ages were not given, the decrease was observed with increase in body weight (from 15 to 90 kg) as the animals aged. In the cattle the decrease was observed between 12 and 24 months of age. In the rat decrease was observed between the 6 weeks old animal and the adult. The loss of fibres in those cases was considerable and could not be ignored.

One argument against the indirect method of counting muscle fibres employed in this study and those of Montgomery (1962), Staun (1963) and Bendal & Voyle (1967) is that all fibres may not be counted, or that there may be an over-estimation of the number of muscle fibres present. Rayne & Crawford (1975) counted directly all the muscle fibres present in the medial pterygoid muscle of the rat and still observed a significant decrease in the total number of muscle fibres present. The indirect method of muscle fibre counting of Jimnez *et al.* (1975) employed in this study was compared to the direct method (counting all fibres) and found to be as accurate as the latter (Jimnez *et al.* 1975). The indirect method employed in this study, as well as in the other studies cited cannot therefore be responsible for the very significant decrease observed.

The actual factors responsible for the loss of muscle fibres with age is not certainly known. Rayne & Crawford (1975) could offer no explanation for the significant fall in the number of muscle fibres of the medial pterygoid muscle of the rat between 6 weeks and the adult stage. Staun (1963) stated that reduction in the number of muscle fibres with age was related to an increase in cross sectional area of the

muscle. Bendal & Voyle (1967) stated that as muscles grow to maturity the increase in cross sectional area of individual fibres is out of proportion to the increase in total muscle area. According to these authors, as the muscle grows larger and larger a stage is reached when some of the fibres become starved of nutrients and oxygen and degenerate, reducing the total number of muscle fibres present. They estimated that as many as 50 % of the muscle fibres may be lost by 24 months of age in the longissimus dorsi and semitendinosus muscles of cattle. They further stated that loss of muscle fibres in this manner could also be deduced from the growth curves published by Joubert (1956) for the lamb, and by Goldspink (1962, 1964) for the mouse. In support of their hypothesis, Bendal & Voyle (1967) stated that they observed distinctly stained macrophages around presumably degenerating muscle fibres in 18 to 24 months old cattle: they did not see such cells in younger animals. Bendal & Voyle (1967) tend to be supported by the recent findings of Curry (1976) who studied cell renewal in the normal soleus muscle of the cat. Curry indicated that the events in the renewal process were similar to those in myogenesis and regeneration. He observed that degeneration followed by regeneration occurred along the full length of the normal soleus muscle. He also observed that the frequency of degeneration increased with age. There is therefore the possibility that in certain muscles the rate of degeneration may come to exceed the rate of regeneration, with consequent reduction in fibre numbers. Use and disuse may be involved here. The present study has established that there is a considerable decrease in the total number of muscle fibres present in the pectineus muscle of dog and that there is loss of all three (histochemical) muscle fibre types. The factors responsible, however, remain unknown.

SUMMARY

The total number of muscle fibres, as well as the numbers of Type I, Type II and intermediate muscle fibre types, in transverse sections of the the pectineus muscles of dogs of 2 and 12 months of age was determined by an indirect fibre counting method. The total numbers of fibres in all muscles decreased very significantly (by about 32 %) in both sexes between 2 and 12 months of age. The decrease resulted from loss of about 20 % of the Type I, 39 % of the Type II and 73 % of the intermediate muscle fibres.

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