Navez, M., J. Dahan, V. de Bethune and G. Maréchal (1987)
Les myosines natives des muscles masticateurs du chat par électrophorèse en milieu non-dissociant
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Native myosins of the masticatory muscles of the cat using non-dissociating gel electrophoresis

(translated from French)

INTRODUCTION

Since dento-facial orthopedics requires good knowledge of the functional aspect of the masticatory system, we had to find an investigation technique capable of identifying the different muscle myosins.

Electrophoresis in a non-dissociating medium, that is to say, not altering the myosinic structure, responds particularly well to this objective.

The cat, by its movements of elevation and closure along a pure hinge axis, such as a pair of scissors (Figure 1), is a masticatory model apparently simpler than that of man, in which the different masticatory functions are mixed, making their interpretation more delicate. The histological AT-Pase techniques according to ROWLERSON (1983) have demonstrated in the masticatory cat particular fibers called IIm, the topography and percentage of which vary according to the location of the specimen and the animal under study, It was therefore interesting to perform a physiological rather than anatomical dissection and to see if the myosins are different, as are the muscular fibers.

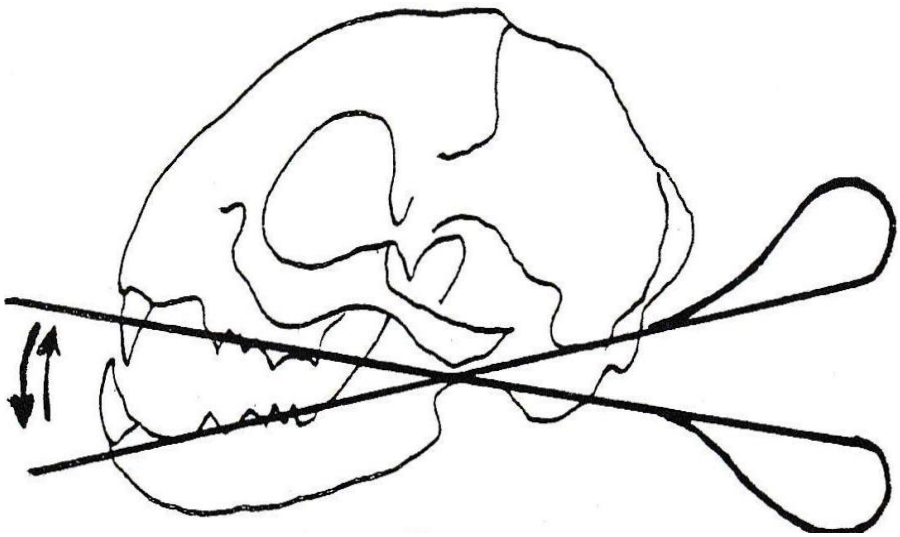

Figure 1

Crane of cat (lateral view). The condyle acts as a pure center of rotation.

MATERIAL AND METHODS

1 - Samples

Five cats of 4 to 5 months, after premedication to Rompun (xylasin: 0.5 mg / kg subcutaneous) were euthanized by intrapulmonary injection of Nembutal (pentobarbital sodium 60 mg / ml: 1 ml / kg). The masseter was dissected into superficial, medium and deep planes (Figure 2a). The superficial plane was further divided into anterior and posterior portions. In the temporal we have retained the following beams: the accessory beam (typical of the cat), the coronoidal insertion in the anterior or rostral region, the middle part and finally the posterior region.The internal pterygoid sample was collected 2 cm higher than its insertion inferior to the internal face of the ramus so as to be not confused with the fibers coming from the masseter. It was at its condylar insertion that the external pterygoid was taken. The digastric (Fig. 2 b) was finally obtained in its middle part. For the other muscles (extensor digitorum longus (E.D.L.), soleus and psoas, the samples were made in full fleshy mass.

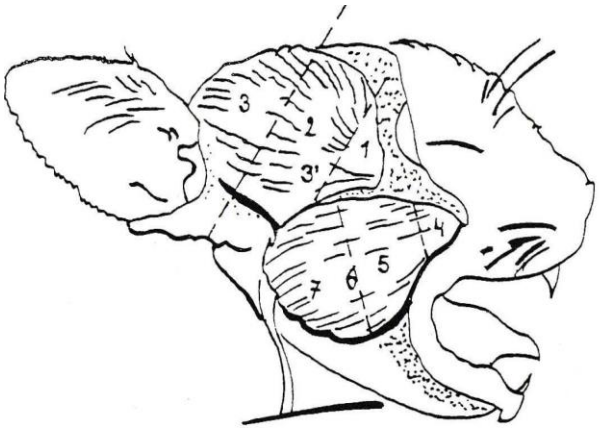

Figure 2a

Cat head (side view). *Sample collection locations*.
Temporal: coronoid insertion on the coronoid process.
Temporal anterior: 1 anterior superficial masseter: 4
Temporal mean: 2 medium superficial masseter: 5
Temporal posterior: 3, medium masseter: 6
Temporal accessory beam: 3 ' deep masseter: 7

Figure 2b

Cat mandible (lower view).
The cross indicates the location of the digastric sample.

2 - Muscle extracts

Each specimen was first weighed and then ground in a mortar in the presence of liquid nitrogen. The powder was extracted in 4 volumes of GUBA-STRAUB solution for 20 minutes on ice. The GUBA-STRAUB solution contains: 0.3 M NaCl, 0.1 M NaH2Po4, 0.05 M Na2HPo4, 1 mM MgCl 2, 10 mM Na 4 P 2 O 7, 0.1% NaN 3, pH 6.5, 0.10 / 0 of 2-beta Mercaptoethanol, to be added immediately before use The suspension was then centrifuged for 10 minutes at 20000 trm, 2°C.

The supernatant mixture having a volume of glycerol is usable immediately, which is preferable, or stored until use at -20 ° C.

The muscle extract of myosin was used without further purification for polyacrylamide gel electrophoresis according to the technique described by HOH et al. (1976) and d'ALBIS et al. (1979) with some modifications.

The preparation of gels, the course of electrophoresis and the measurements of the mobility and proportion of isozymes have been described in detail elsewhere (MARÉCHAL et al., 1984).

3 - Mobility

The mobility of myosins is calculated relative to that of SM2 (slow myosin of rat), set at 100%. In practice, it is evaluated in comparison with the mobility of the myosin of taenia coli (fixed at 147%), added to the myosin extracts as an internal standard of mobility

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RESULTS

FIG. 3 shows the electrophoretogram of the eleven samples of the elevating masticatory muscles compared to a step-down muscle, the digastric and two peripheral skeletal muscles, E.D.L. and the psoas. Cat Soleus has been added for comparison. In each case, the fastest band is the myosin of taenia coli (smooth muscle in guinea pig) used as an internal standard.

The elevating muscles of the mandible show one or two isomyosins, while the other specimens show one (soleus), three (psoas) or four (E.D.L.).

Figure 4 shows the densitometry of the five closure muscles compared to the adult soleus and E.D. L. The right peak corresponds to the smooth muscle. Three elevating muscles show a single peak (a, b, c), the other two peaks (d, e); E.D.L. is characterized by four peaks, soleus by one.

Table I reports the mobility of myosin muscle elevators, called JM1 and JM2 as a function of their decreasing migration speed. All elevating masticatory muscles contain an isomyosin that migrates to the velocity (115 ± 1). Six of these had a slower second myosin (104 ± 1). The other peripheral muscles (EDL, psoas, soleus) contain the three typical fast isomyosins: FM1, FM2, FM3 (see d'ALBIS et al., 1979, HOH and YEOH, 1979) and the two slow isomyosins SM1 and SM2. The mobilities are similar to those observed by MARÉCHAL et al., 1984.

Table II analyzes the proportions of the isomyosins recorded by densitometry and this one relative to the others, obviously excluding the myosin from the taenia coli. When the masticatory myosins JM1 and JM2 are present, they are present in equal proportions except in the posterior portion of the surface masseter, the ratio JM1 / JM2 is 2/1. In the peripheral muscles, the distribution of the different myosins is much more stable.

The standard error of the averages is significantly greater in the masticatory muscles, elevators than in the peripheral muscles. The masticatory muscles vary more according to the individual examined than do other skeletal muscles

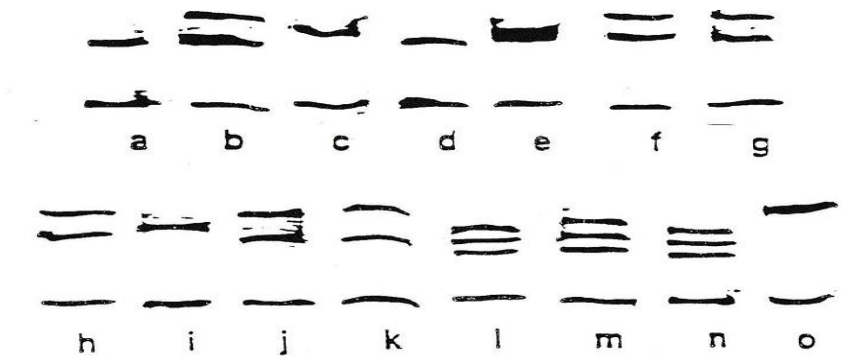

Figure 3
Electrophoresis in pyrophosphate gel of cat isomyosins.

Only the myosin bands are shown. In all specimens, the lowest band corresponds to the smooth muscle of the guinea pig taenia coli. Electrophoresis temperature: 4 deg centigrade

Temporal (a. - e.):
a: Accessory bundle
b: Coronoid insertion
c: anterior
d: medium
e: posterior.

Masseter (f.-i.):
f: superficial anterior
g: superficial posterior
h: medium
i: deep

j: Internal Pterygoid
k: External Pterygoid
l: Digastric
m: Psoas
n: E.D.L.
o: Soleus

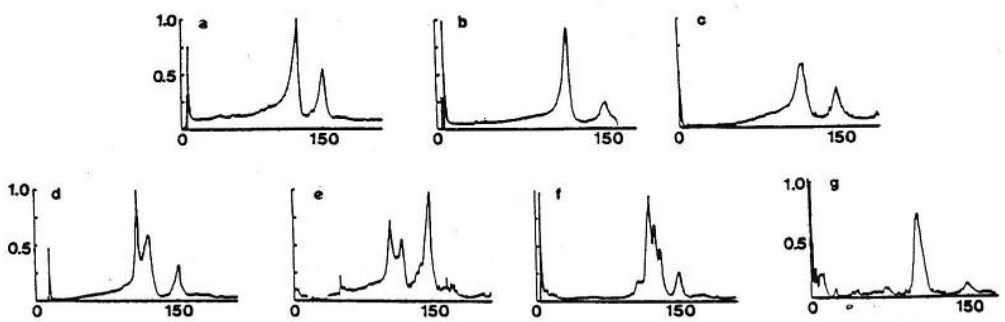

Figure 4
Profile of cat myosins. The gels a, c, d, j, k, o in Figure. 3 were scanned at 536 nm.
Ordinates: absorbance in arbitrary unit, *Abscissa*: mobility (mobility of taenia coli of guinea pig = 147).

a: Temporal accessory
b:Anterior Temporal
c: Middle Temporal
d: Internal Pterygoid
e: External Pterygoid
f: E.D.L ..
g: Soleus

The peak (right) corresponds to the myosin of the smooth muscle: taenia coli. The peak (left) corresponds to JM1 in (a), (b) and (c). The doublet in (c), (d) and (e) corresponds to JM1 (right) and JM2. E.D.L. (F) shows the three fast myosins FM1, FM2 and FM3: the right triplet and a slow myosin
called SM1 according to BECKERS-BLEUKX and MARÉCHAL1 (1986).
Soleus (g) shows a single band that corresponds to another variety of slow myosin, called SM2
according to BECKERS-BLEUKX and MARÉCHAL 1 (1986).

ISOMYOSINS	SM2	SM1 JM2	FM3 JM1	FM2	FM1
CAT Muscles					
Temporal					
Accessory bundle		—	116		
Anterior coronoid		104	115		
Anterior		—	116		
Medium		—	115		
Posterior		—	114		
Masseter					
Anterior superficial		106	115		
Posterior superficial		104	112		
Medium		104	115		
Deep		—	115		
Pterygoïdien					
ext.		103	113		
int.		106	116		
Digastric			115	119	125
Psoas			115	121	125
E.D.L.		104	113	118	123
Soleus	101				
Soleus rat*	100	107			
E.D.L. rat*			118	122	125

Table I

Mobilities of cat isomyosins, n=4, standard deviation=1.0

*The mobilities of rat isomyosins from MARÉCHAL et al 1984

ISOMYOSINS	SM2	SM1 JM2	FM3 JM1	FM2	FM1
CAT Muscles					
Temporal					
Accessory bundle		0	100		
Anterior coronoid		44 ± 4	56 ± 4		
Anterior		0	100		
Medium		0	100		
Posterior		0	100		
Masseter					
Anterior superficial		46 ± 4	48 ± 5		
Posterior superficial		29 ± 6	71 ± 6		
Medium		45 ± 6	55 ± 6		
Deep			100		
Pterygoïdien					
ext.		51 ± 8	49 ± 8		
int.		42 ± 9	58 ± 9		
Digastric			55 ± 1	29 ± 1	18 ± 3
Psoas			49 ± 4	38 ± 1	15 ± 3
E.D.L.		5 ± 1	36 ± 5	33 ± 2	26 ± 4
Soleus	100				
Soleus rat*	71 ± 4	26 ± 3			
E.D.L. rat*		31 ± 3	34 ± 2	35 ± 3	

Table II

Isomyosin contents in % of total myosin, n=4

*data from MARÉCHAL et al. 1984

DISCUSSION

The masticatory muscles in young cat have one or two isomyosins (JM1 and JM2) detectable by electrophoresis of undissociated myosins. Rapid muscles, on the other hand, such as psoas, have three, the mixed muscles, such as E.D. L. (*Extensor digitorum longus*), four. The digastric, a muscle of mandibular opening, is characterized by three fast myosins, exactly like psoas.

JM1 is the fastest myosin: its electrophoretic mobility of 115 is similar to that of FM3, the slowest of the three isomyosins typical of fast muscles. However, it cannot be assumed that JM1 and FM3 contain the same isomyosin, as ROWLERSON et al. (1981) isolated an isomyosin from the fast muscle myosin by its heavy chains; These authors called it "Super-Fast Myosin" because of its important ATPase activity. In the posterior temporal we observed only one isomyosin, JM1.

JM1 is therefore this very rapid myosin which could exist in the Ilrn fibers (MASCARELLO et al., 1979, ROWLERSON et al., 1981, TAYLOR et al., 1973).

JM2 is a slower isomyosin. Its electrophoretic mobility (104 ± 1) is identical to that of SM1, but significantly higher than SM2 (mobility = 100), which is the specific myosin of type I fibers in the soleus of rats and cats. The meaning of SM1 is unclear. It was observed in the soleus of rat and dog (1), in the E.D.L. of rat and rabbit (BECKERS-BLEUKX and MARÉCHAL, 1986) and in rabbit ear muscle (MORARU et al, 1984). Its proportion is low in these muscles, about 5 to 20% of total myosin. To the contrary, the proportion of JM2 is high (50%) in several muscle elevators of jaw (Table II). JM2 may be a second slow form of slow myosin, perhaps more specialized in maintaining mandibular posture tone.

The masticatory myosins are not distributed evenly, even in the anatomically well defined muscles such as the temporal or the masseter. The temporal contains only JM1, except for its anterior portion near the coronoid apophysis. The masseter is rather mixed with two isomyosins in an almost equal proportion, except in the deep proportion which contains only JM1.

Pterygoid muscles have the same myosinic profile as the superficial masseter.

It may be remarked here that the different bundles of the masseter and the temporal have not the same physiological action. The temporal and the deep masseter are topographically better implanted for the bite in strength. The anterior temporal has its coronoid insertion and the other masseterine regions have an insertion on the jaw with a less favorable line of action. We therefore conclude that the cat's masticatory muscles have two isomyosins that can be isolated by electrophoresis, each with a specific physiological function: JM1 is a rapid myosin used in the immediate and powerful contraction of the jaw while JM2 is a slower myosin, specialized in maintaining the tone of posture to keep the mouth of the animal closed. This is a conclusion similar to that of ROWLERSON et al. (1983): The digastric as a masticatory-buckling muscle does not have the same isomyosins. With its three isomyosins very close to the psoas, it is more similar to peripheral muscles. ROWLERSON has not found there any fibers Ilm

(1) *Note added by the translator,(June 2017).* This is a mistake, since dogs have no soleus. In the paper of BECKERS-BLEUKX and MARÉCHAL (1986) there are no data on dog soleus, but there are data on dog heart ventricle: it contains three isomyosins, V1, V2 and V3, and the mobility of V2 is very similar to that of SM1.

Thanks

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ABSTRACT

Two isomyosins (JM1 and JM2) have been isolated by pyrophosphate-polyacrylamide gel electrophoresis from jaw-closing muscles of cats aged 4 months. JM1 shows a high electrophoretic mobility (115 ± 1, rat soleus = 100) and is probably the superfast jaw myosin: it is pure in temporal and in deep masseter.

JM2 shows a low electrophoretic mobility (104 ± 1): it is similar to the slower isomyosin observed in extensor digitorum longus, but different from the slow isomyosin of soleus. The distribution of the two isomyosins in the various bundles of jaw muscles indicates that JM1 could be more related to phasic, biting activities, whilst JM2 could be more involved in the tonic closure of the mouth

KEYWORDS

Cat– Muscles Jaw Temporalis Masseter Digastric Pterygoid - Myosins –JM1 JM2.

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