Acetylcholinesterase molecular forms in the fast or slow muscles of the chicken and the pigeon

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Molecular forms of acetylcholinesterase (AChE) were examined in various skeletal muscles of the chicken and the pigeon. In chicken pectoralis m., AChE was found to be restricted to endplate containing segments, and no asymmetric form could be detected in aneural samples. In the chicken muscles studied, a relation has been established between globular (G_1,G_2,G_4) forms or asymmetric (A_8,A_{12}) forms, and muscle fibre types. Asymmetric forms are preponderant in fast-twitch muscles, whereas in slow tonic muscles 80% of the AChE activity is due to globular forms. However, comparison with pigeon muscles shows that AChE chicken muscle patterns may not be generalized.

AChE molecular form *Muscle* *Chicken* *Pigeon*

1. INTRODUCTION

It is well known that acetylcholinesterase (AChE, EC 3.1.1.7) is a very polymorphic enzyme [1] presenting molecular forms classified as globular (monomer G_1, dimer G_2 and tetramer G_4) and asymmetric or collagen-tailed forms (containing 1, 2 or 3 tetramers: A_4, A_8 and A_{12}). The complexity of such a polymorphic system raises numerous questions [2], amongst others, the relationship between molecular forms and their muscular localization which is of the utmost importance for the understanding of nerve muscle interactions.

The cellular localization of the different forms of AChE has been studied in [3–5]. In rat and rabbit muscles, the A_{12} form – which represents the major asymmetric collagen-tailed form – is localized exclusively at the neuromuscular endplate, whereas in chicken [6] and in human muscles [7] these forms would appear to be distributed over the entire muscle fibre. Otherwise, the distinctive patterns of collagen-tailed and globular forms in chicken muscles is different: collagen-tailed forms are more abundant in fast-twitch muscles whereas in slow tonic muscles globular forms are dominant [8]. In order to know if this can be generalized, we here report results of experimental studies on various chicken and pigeon muscles, related to the cellular localization of AChE molecular forms, and to the importance of muscle activity for their distribution. Our findings indicate that:

(i) Contrary to what was observed in previous studies, AChE is restricted in the pectoralis m. of the chicken to endplate-containing segments, and no asymmetric form is detected in aneural samples;

(ii) The AChE molecular form pattern of the different chicken muscle types may not be generalized to other species.

2. MATERIALS AND METHODS

2.1. *Choice and preparation of muscle samples*

Five muscles of 4-week old chickens, known to be homogeneous and constituted of only one type of fibre have been studied [9]:

(i) Two glycolytic fast-twitch muscles (Fibre II B type): pectoralis major m. and posterior latissimus dorsi m. (PLD);

(ii) One oxidative fast-twitch muscle (Fibre II A type): adductor longus;

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(iii) Two oxidative slow tonic muscles (Fibre I type): anterior latissimus dorsi m. (ALD) and the posterior part of adductor magnus m. [10].

Two muscles of the pigeon were also used: the fast-twitch pectoralis major, essentially constituted of fibre II A type with a few fibre II B types [11], and the slow tonic ALD (fibre I type).

Immediately after death, muscles were removed and either frozen at −80°C for AChE molecular form characterization, or used immediately for histochemical stainings.

2.2. *Staining and dissection of endplate-free segments of pectoralis muscle of the chicken*

Fibre bundles of pectoralis m. were dissected in 0.01 M malic acid/NaOH (pH 7.0) buffer, with $CuSO_4$ 0.002 M, glycine 0.008 M and acetylthiocholine iodide 0.004 M, as in [14]. At room temperature, white spots corresponding to AChE staining of motor endplates appeared at regular intervals on fibre bundles. The dissection (about 30 min) under binocular lens allows the separation of endplate-containing segments (neural zone) and endplate-free segments (aneural zone). Samples (10–20 mg) of such dissected tissues were stored frozen at −80°C until used.

2.3. *AChE assay*

Muscle samples were homogenized at 4°C in a glass-to-glass Potter homogenizer with 10 vol. of extraction medium (Tris–HCl, pH 7.2, 0.01 M buffer, NaCl 1 M, Triton X-100 1%, EGTA 1 mM, containing aprotinine 5 IU/l and benzamidine 1 mM as antiproteolytic agents [15]). After centrifugation at 30000 × *g* for 20 min, 80–150 μl of the supernatant were layered on 10.25% (w/v) sucrose gradients in extraction medium, and centrifuged in a Beckman SW 41 rotor, at 40000 rev./min for 20 h, at 4°C. AChE was detected in each of the 45 or so fractions from each gradient as in [16], using 0.1 mM iso OMPA, an inhibitor of non-specific cholinesterase. The apparent sedimentation coefficients of AChE forms were calculated by comparison with those of *Escherichia coli* β-galactosidase (16 S) and alkaline phosphatase (6.1 S).

3. RESULTS

3.1. *Distribution of AChE molecular forms according to chicken muscle fibre types (fig.1,3)*

In each of the studied muscles, 5 molecular forms were separated, with sedimentation coeffi-

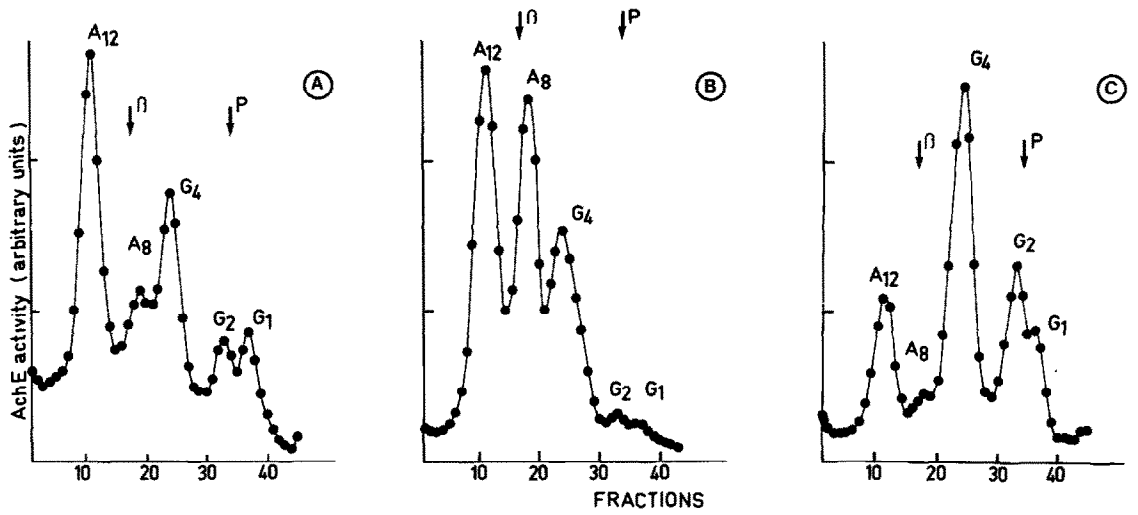

Fig.1. Sedimentation patterns of AChE in the chicken PLD m(A), adductor longus (B) and posterior part of adductor magnus (C). Activities are plotted on an arbitrary scale but profiles corresponding to each muscle have been normalized so that proportions of each form in the 3 muscles are directly comparable. β = β-galactosidase (16 S); P = alkaline phosphatase (6.1 S).

cients of 4.3 S (G_1), 6.3 S (G_2), 11.8 S (G_4), 15.1 S (A_8) and 19.3 S (A_{12}). Their relative proportions were estimated from the sedimentation profile of each muscle and are in agreement with those obtained by [8]. The sedimentation profile of AChE molecular forms is characterized by the importance of collagen-tailed *vs* globular forms in fast-twitch muscles (fig.1 and 3B) whatever their oxidative (Type IIA fibres) or glycolytic (Type IIB fibres) metabolic properties may be. The two slow tonic muscles (fig.1,3A) have both a similar sedimentation profile of AChE molecular forms, independent of their anatomical situation. Compared to the pattern of AChE fast-twitch muscles, it is characterized by the predominance of globular *vs* asymmetric forms.

3.2. *Localization of AChE molecular forms in the chicken pectoralis m.*

Total AChE activity of pectoralis m. is very low in endplate-free segments (<0.1 nM.min^{-1}.mg protein^{-1}) in comparison with AChE activity of total fibres (0.4 nM.min^{-1}.mg^{-1}) and particularly endplate-containing segments (1.2 nM.min^{-1}.mg^{-1}). Comparison of the sedimentation profiles of the different dissected fibre segments is in agreement with these data (fig.2). The sedimentation profile of endplate-free segments roughly follows a baseline. However, although it is possible to distinguish the G_4 and a light trace of G_1 and G_2 forms, an additional period of incubation of 24 h of the different fractions does not change the sedimentation profile of aneural segments. In endplate-containing segments, we observe 5 molecular forms and 70% of the total AChE activity is represented by asymmetric forms. Thus, the essential part of pectoralis AChE activity is concentrated at the neuromuscular junction, and collagen-tailed forms are specifically localized at this level.

3.3. *AChE molecular forms in the pigeon pectoralis m. and ALD m.*

Compared with chicken muscles, pectoralis m. and ALD m. of the pigeon differ in their relative proportions of various AChE forms as in their apparent sedimentation coefficient (fig.3).

In the slow tonic ALD m., globular forms account for 58% of AChE activity and are represented mostly by the G_1 and G_4 forms, with a very low level of G_2. The proportion (42%) of collagen-tailed forms is more important than in the chicken, the A_{12} form occurring for 33% of total AChE activity.

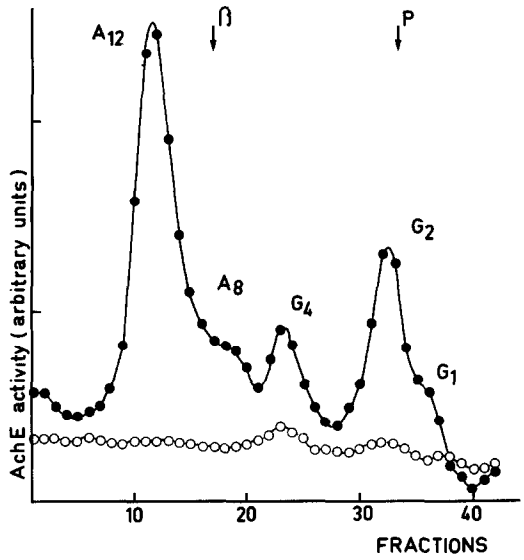

Fig.2. AChE sedimentation patterns of endplate-containing (●—●) and endplate-free (○—○) segments of the chicken pectoralis m. Same arbitrary scale used for both profiles.

In the fast-twitch pectoralis m., globular forms account for 75% of AChE activity, and are represented essentially as for ALD m. by the G_4 and G_1 forms. The percentage of asymmetric forms (25%) is weak compared to the 64% observed in the chicken pectoralis m.

Otherwise, one of the most striking results of this comparative study is the difference in the apparent sedimentation coefficient of some pigeon and chicken AChE molecular forms. As shown in table 1, the apparent sedimentation coefficients of the soluble G_1 and G_2 form are quite identical in both birds, but the membrane-linked G_4, A_8 and A_{12} forms have heavier sedimentation coefficients in pigeon than in chicken.

4. DISCUSSION

The distribution of the collagen-tailed and globular forms of AChE along the muscle fibres varies according to the animal species and physiological state. In normal adult rat muscle, the

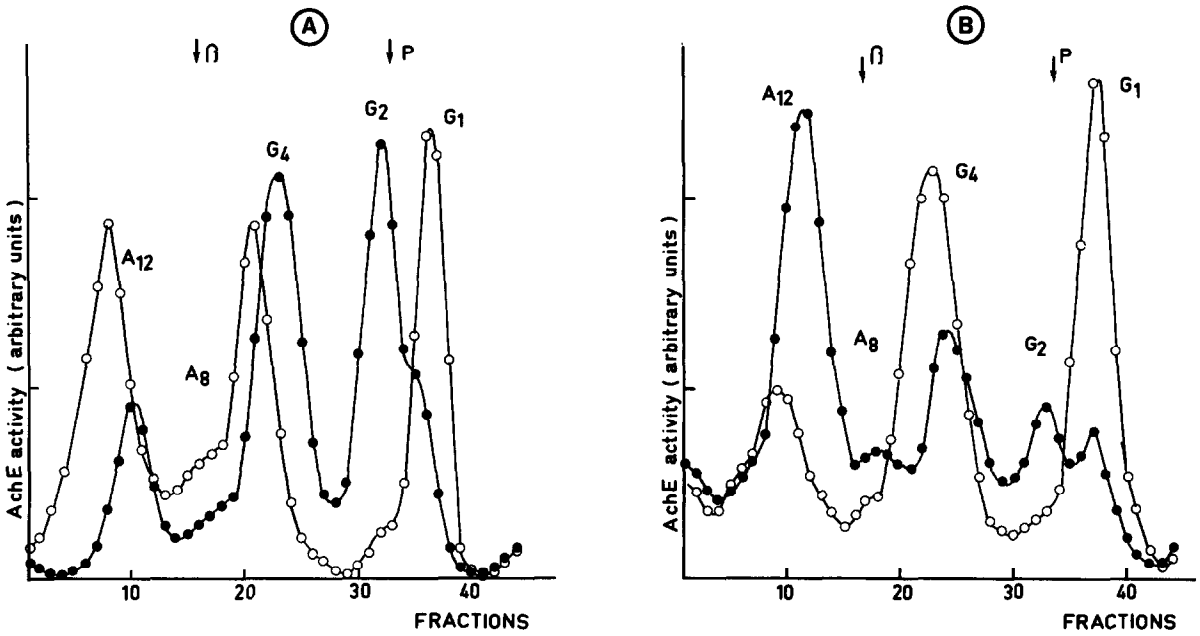

Fig.3. AChE sedimentation patterns of ALD and pectoralis muscles. (A) ALD m. of the chicken (●—●) and the pigeon (○—○). (B) Pectoralis m. of the chicken (●—●) and the pigeon (○—○). In both species, activities of the homogenates are 5-times higher in ALD m. than in pectoralis m. But the scale corresponding to AChE profiles has been normalized so that proportions of each form are directly comparable.

A_{12} form, which represents the major collagen-tailed form, is localized exclusively in the endplate region [17], whereas in rat embryo [18] and human muscle this form is distributed over the entire muscle fibre [7]. Our study on the pectoralis m. of the chicken shows that collagen-tailed forms are localized only in endplate-containing segments. The presence of collagen-tailed AChE has been considered as an indicator of neuromuscular interactions because its appearance during embryogenesis coincides with the establishment of neuromuscular contacts and because after denervation, the A_{12} form disappears from rat and chicken muscles, but only from the fast-twitch muscles of rabbits [5].

The regulation of AChE molecular, particularly

Table 1

Sedimentation coefficients of chicken and pigeon muscle forms of AChE

	A_{12}	A_8	G_4	G_2	G_1
Pigeon	20.8 ± 0.3 S[a] (11)	16.2 ± 0.2 S[b] (10)	13.5 ± 0.3 S[c] (11)	6.4 ± 0.3 S (6)	4.3 ± 0.6 S (11)
Chicken	19.3 ± 0.5 S[a] (10)	15.1 ± 0.4 S[b] (8)	11.8 ± 0.5 S[c] (10)	6.3 ± 0.3 S (9)	4.3 ± 0.4 S (9)

Mean ± standard deviation; numbers in parentheses, values calculated from pectoralis m. and ALD m. Significant differences ($p < 0.001$) between each of the chicken and pigeon AChE forms (Student's t-test). Other differences not significant. The sedimentation coefficients indicated correspond to the apparent values, obtained in both species under our experimental conditions

asymmetric, forms thus depends on the species and on the fast or slow nature of skeletal muscle. This is corroborated when comparing the AChE pattern to the fibre type composition of the chicken and pigeon muscles. The relation between fibre type and AChE profile of chicken muscles (fig.1,3) cannot be extended to the corresponding ones in the pigeon. In both species, the muscles studied have the same fast (pectoralis) or slow (ALD) contractile properties, but differ in their general activity. Other physiological characteristics, such as sustained or temporary muscle activity, could thus be involved in the regulation of AChE molecular forms.

Indeed, numerous data confirm the importance of motor activity on AChE synthesis and on the level of asymmetric forms, particularly the A_{12} form. The paralysis by a curare-like drug of chicken embryos reduces both the histochemical AChE-staining of endplates [19] and the level of the A_{12} form [20]. In cultures of rat muscle cells from 18-day old embryos, appearance of the biosynthesis of the A_{12} form seems to be correlated with their spontaneous contractile activity, since both are reversibly blocked by TTX [21]. However, inhibition of muscle culture contractions by Veratridine (which maintains the sodium channel in the open state) induces a 15-times higher specific activity of the A_{12} form as compared to contractile control cultures [22]. This observation demonstrates that contractile activity per se would not be necessary for the synthesis of the heaviest asymmetric form of AChE, but that ionic fluxes through the membrane probably play an essential role in this regulation. Thus, the distinctive AChE patterns in pigeon and chicken muscles would appear to arise less from the type of activity (fast or slow, sustained or temporary) than from different physiological properties of muscle membranes.

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