DISAPPEARANCE OF THE 'ENDPLATE' FORM OF ACETYLCHOLINESTERASE FROM A SLOW TONIC MUSCLE

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1. Introduction

Acetylcholinesterase (AChE, EC 3.1.1.7) and pseudocholinesterase (ψChE, EC 3.1.1.8) have been shown to exist in muscles and in other tissues in multiple molecular forms [1–5]. In chicken fast-twitch muscles, for example, the occurrence of two light forms (L_1 and L_2, $s_{20,w}$ = 4.7 S and 6.6 S), a medium (M, 11 S) and two heavy forms (H_1 and H_2, 14.7 S and 20 S) of AChE have been followed through development to maturity [6,7]. In mammalian muscles, similar AChE forms (~4 S, 6 S, 10 S, 13 S and 16 S) were described [1–4,8]. The 16 S mammalian and the 20 S avian H_2 forms are widely accepted as constituting the AChE at the motor endplates, since they are not found in non-endplate zones of muscles and since they disappear on denervation [1–3,8,9] (and a similar conclusion has recently been drawn for their H_1 forms [4]); however, in human muscle [10] some H AChE has been described as being also outside the endplates. The mammalian 16 S, avian 20 S and fish electric organ 16.5 S forms of AChE have been characterised as comprising a collageneous tail to which 12 catalytic monomers are attached in clusters [4,5]. The tail is thought to anchor this structure to the basal lamina [11], the whole being designed for function external to the synaptic membrane.

ψChE (which differs from AChE in some specificity properties) has recently been shown to exist in a similar set of forms in fast-twitch muscles and ganglia, and the two enzymes appear to be regulated in parallel there [4,7,9]. Again, H_2 ψChE, alone, disappears on denervation [9], indicating an endplate location.

The slow (tonic) muscles, e.g., the avian anterior latissimus dorsi (ALD), possess many endplates per fibre. It has been noted briefly [6] that the proportions of the various AChE forms in the ALD muscle at 60 days are different from those in fast-twitch muscles, the ALD apparently having much less of the H_2 form. We report here a developmental study to show that in these endplates, unlike those of the twitch muscles already discussed, the H forms of AChE and of ψChE disappear with maturation, suggesting that a different molecular architecture holds these enzymes at this type of synapse.

2. Materials and methods

Muscle samples were taken from normal chickens of our colony of the University of California (Davis) 412 strain, and extracted and homogenized as in [7]. Where indicated, perfused muscle was obtained by exhaustive whole body perfusion of saline through the jugular vein.

AChE and ψChe were assayed at 25°C in the presence of the required specific inhibitors [7]. Sucrose density gradient centrifugation analysis was as in [7].

3. Results

The concentration of AChE is much greater than that of ψChE throughout development of the ALD muscle (fig.1). Both, however, show the same pattern of decreasing concentration after hatching. Following the period of rapid growth in these birds (up to 70 days), the concentrations are relatively constant. The apparent decrease seems to be not a loss of enzyme but a dilution artifact due to the increasing size of the growing muscle, since the total amount of

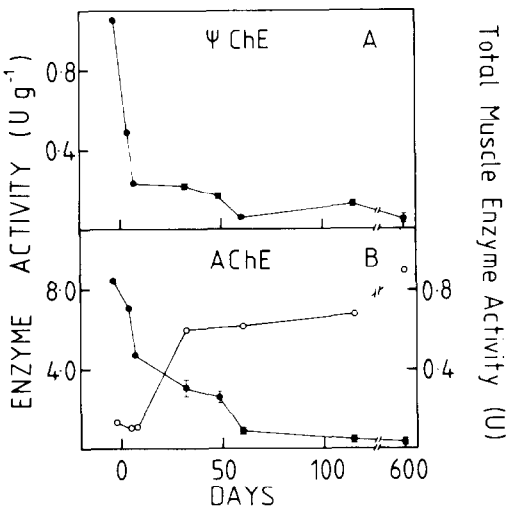

Fig.1. Concentrations of ψChE (A) and AChE (B, ○) in the ALD muscle. The broken line indicates the day of hatching. The total amount of AChE per pair of ALD muscles (○—○) is also shown in (B). Points up to 7 days represent the pooled samples from 4 birds; at later ages the mean for 2–5 birds, with bars for SEM, is shown.

AChE per ALD muscle rises notably in the same period (fig.1B).

Late in embryonic development (fig.2A) all forms of AChE are present but L_2 and H_2 (as defined in fig.2B) predominate. A very similar pattern is seen at 7 days after hatching (not shown) and at 32 days (fig.2B). By 66 days (fig.2C), however, a substantial decrease in the proportion of H_2 is seen, and this continues during further maturation of the muscle until virtually no H_2 is detected in the adult (fig.2E,F). The various amounts of H_2 shown in fig.2 are believed to be free of artefact due to any preferential degradation of that form since all extractions were made in a complete mixture of protease inhibitors effective for protecting chick muscle AChE.

For ψChE (fig.3), likewise, the 3 main forms are

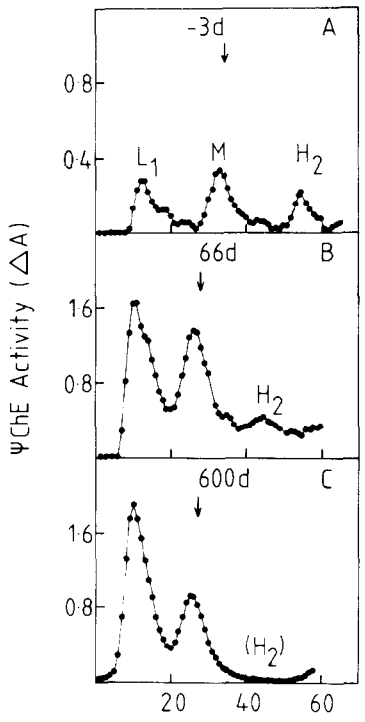

Fig.3. Sucrose gradient profiles for ψChE in ALD extracts. Other details are as in fig.2, except that the assay incubation times were 6 h (A), 48 h (B) and 20 h (C). The $s_{20,w}$ values [7] are 4.0 S for L_1 ψChE, 10.5 S for M, and 19.2 S for H.

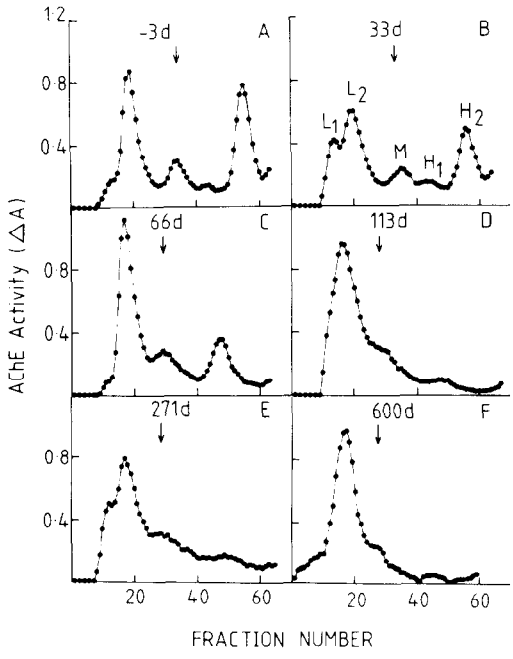

Fig.2. Sucrose gradient profiles for AChE in ALD extracts. The age of each bird is indicated. The designations and observed $s_{20,w}$ values of the peaks are as given in [7] for pectoral muscle. Note the virtual absence of H_2 in D, E and F. Arrows denote the position of the beef liver catalase (11.4 S) marker; horse liver alcohol dehydrogenase (4.8 S) and *Escherichia coli* β-galactosidase (16 S) were also used for calibration [7] of the gradients. The muscle used in C was from a perfused bird. Assay incubation times were 1–6 h.

substantial just prior to hatching, but the H form disappears during maturation, being completely absent in the adult. The muscle shown for 66 days was perfused to remove all traces of blood which has considerable ψChE (but negligible AChE) and where we have found primarily M ψChE (but no H). Perfusion was not feasible in the embryo and the relatively high proportion of M ψChE therein may, in part, be due to such plasma contamination. Therefore, L_1 and M ψChE are always present as intrinsic forms in the ALD muscle, but in mature ALD the H_2 form vanishes.

4. Discussion

These slow tonic muscles of the adult chicken provide the first known case of a vertebrate skeletal muscle lacking a 'heavy' form of AChE. It is clear that these muscles are initially equipped, like others, with that form at their endplates, since it is present at early stages, but it disappears during the growth of the bird. ψChE is regulated in a parallel manner during maturation.

Although the concentration of AChE in the ALD muscle declines during growth due to the increase in the size of the fibres, final levels are nevertheless appreciable, and a similar decline in concentration with growth is also found in the chicken fast-twitch muscles that have been studied [7], where H_2 AChE does not disappear. On the contrary, in the fast-twitch muscles H_2 is the major form present at all times after hatching, its proportion increasing further with age [7]. Despite the AChE concentration decrease in the ALD muscle, the total quantity of AChE there is seen to increase as the bird matures (fig.1B). The total of H AChE, however, is negligible at 5 months and later, being $\leqslant 3\%$ of the total AChE. Yet there is undoubtedly AChE located at the endplates, since several cytochemical studies show the staining for AChE to be there in the mature chicken ALD [12–15], as does a specific [^{3}H]DFP-labelling method for AChE quantitation at the endplates [16]. It has, indeed, been confirmed in this laboratory that in our birds (160 and 600 days) the endplates, alone, can be seen to be clearly stained (albeit more weakly than in the PLD) by a method [17] for AChE (2.5 h incubation at 22°C; preparations made by J. Lamprecht, not illustrated). Finally, for 224 day chickens total cholinesterase (AChE + ψChE) in micro-dissected single endplates has been reported [18]: the ALD endplate had 26% of the activity in the PLD endplate, a clearly significant concentration.

We conclude, therefore, that the L or M forms (or both) must occur at the endplate. Since neither of these chicken muscle AChE species possess the collagen tail, but are the globular dimeric and tetrameric molecules, respectively [4], they cannot be anchored in the same manner as the H_2 form at twitch muscle endplates. How these globular AChE molecules are arranged at the tonic muscle endplates poses an intriquing question. Those endplates are almost devoid of post-synaptic folds and the terminal size is much smaller than in the fast-twitch muscles [13], while their sensitivity to ACh is much lower [19,20]. They produce graded muscle potentials rather than action potentials; less transmitter is released per impulse and the spontaneous potentials decay much more slowly than at twitch fibre endplates [19–21].

For all of these reasons the disposition of AChE is likely to be less critical than in the twitch muscle endplates, and a different arrangement involving the globular precursor forms of AChE at the endplate appears plausible.

Acknowledgements

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