The Acetylcholine Content and Choline-acetyltransferase Activity in Human Skeletal Muscle

To date there has been a great lack of information on the amount of ACH and CHA activity in human skeletal muscle. However, the assay of the neurotransmitter and of its synthesizing enzyme may allow a better evaluation of the biochemical damage in the motoneurones in the course of some neuromuscular diseases: it is well known that the ACH stores and CHA activity fall to very low levels after section of the motor nerve[1]. In this report the values of ACH and CHA activity in some human skeletal muscles are given.

Methods. Specimens of rectus abdominis m., piramidalis abdominis m. and intercostalis externus m. were obtained during surgical operations from adult patients with no evidence of muscular or neuromuscular diseases. The specimens were excised by two parallel cuts, 1 cm apart, perpendicular to the muscle fibres. The average weight of the samples was 200–400 mg; some were assayed for their ACH content, others for CHA activity.

Estimation of acetylcholine. The excised tissue was weighed and rapidly immersed for 2–3 min in 2 ml of McIlvain citric acid-disodium phosphate buffer pH 4, 0.026 *M*, at 98–99 °C. After cooling, the tissue was homogenized and centrifuged for 20 min at 4000 rpm. The ACH present in 1 ml of the supernatant was precipitated at pH 6.5 by adding choline chloride and ammonium reineckate, as previously described[2]. The precipitate was solubilized in 5 ml Tyrode solution; standards were prepared by adding known ACH amounts to part of the samples, previously boiled at pH 10 for 5 min; then the above-described precipitation procedure was carried out. With this method the recovery is about 100%. The bioassay was performed on guinea-pig terminal ileum kept in 3 ml of oxygenated tyrode solution plus diphenhydramine $2 \cdot 10^{-8}$, and morphine $5 \cdot 10^{-8}$, at 30 °C.

Estimation of choline acetyltransferase. The specimens were plunged into anhydrous acetone at 0 °C (100 mg wet tissue/ml) and homogenized; then 0.15 ml of the suspension (equal to 15 mg of tissue) was laid on a disc of Whatman No. 50 paper (10 mm diameter), placed on a porous filter connected with a water vacuum pump. In this way dry acetonic powder may be obtained on the paper within a few seconds. The procedure is carried out in a refrigerated room at 0–2 °C. The CHA activity of the samples was measured according to BULL et al.[3]. The discs were incubated for 1 h at 38 °C in 0.5 ml of reaction mixture previously kept at the same temperature for 15 min. The enzymatic reaction was stopped with ml 0.2 HCl 0.33 *N*; the samples were buffered and diluted to 5 ml with frog Ringer solution.

The bioassay was performed on eserinized frog rectus abdominis muscle against suitable standards. For further evaluation of the biochemical findings, anatomical data were needed; we therefore also measured the average length of the muscle fibres in the whole muscle and the average diameter of the fibres and their number/cm² in microscopic sections.

Results. The amounts of ACH and CHA present in only one endplate were calculated assuming that: (a) the possible presence of the endplate in every muscle fibre of the specimen (1 cm long) is inversely related to the length of the whole fibre; therefore the number of fibres counted in 1 cm² of the transverse section of the muscle was divided by the length of the muscle itself; (b) the amount of ACH and CHA present in the motor nerve endings is about $^2/_3$ of the total amount found, because of the nervous fibres present in the specimens[4]. The results and their elaboration are summarized in the Table.

The most prominent comment concerns the ratio between synthesizing activity and neurotransmitter stores. It is evident that the ratio is nearly the same in the rectus abdominis m. and in the intercostalis externus m., while it is appreciably lower in the piramidalis abdominis m. Taking into account the physiological work of these 3 muscles, it may be suggested that the higher the ratio, the higher is the neuromuscular activity.

Anatomical data and normal values of ACH and CHA activity in 3 human skeletal muscles

Muscle	Average diameter μ	Average number of fibres/cm²	Average length of fibres, cm	Endplates per cm³ (fibres/length)	ACH *ng*/g fresh tissue	ACH/p*M* per endplate	CHA activity *ng*/g fresh tissue/h	CHA/p*M* per endplate	CHA/ACH ratio
Intercostalis externus m.	55	40,000	5.6	7.142	131.2 ± 37.2[a] (12)	0.067	10,700 ± 2070[b] (9)	5.4	81.5
Rectus abdominis m.	30	97,000	13.5	7.190	85.5 ± 35.3 (10)	0.043	7200 ± 2700 (8)	3.6	84.2
Piramidalis abdominis m.	32	90,000	5.2	17.307	110.7 ± 29.3 (12)	0.023	7200 ± 2800 (6)	1.5	65

[a] This value differs significantly from that of Rectus abdominis m. at a level of $P < 0.01$. [b] This value differs significantly from that of Rectus abdominis m. and Piramidalis abdominis m. at a level of $P < 0.02$. In brackets the number of experiments.

Riassunto. Sono descritte le tecniche per la determinazione della ACH e attività CHA nel muscolo umano normale. I risultati indicano che il rapporto fra capacità sintetizzante e neurormone è più alto in muscoli con maggior impegno funzionale.

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[1] C. O. HEBB, J. Physiol. *163*, 294 (1962).
[2] L. BEANI, C. BIANCHI, and F. LEDDA, J. Physiol. *174*, 172 (1964).
[3] G. BULL, C. O. HEBB, and D. RATKOVICH, Biochim. biophys. Acta *67*, 138 (1963).
[4] C. O. HEBB, C. KRNJEVIĆ, and A. SILVER, J. Physiol. *171*, 504 (1964).