Do peripheral nerves contain a factor inducing acetylcholine sensitivity in skeletal muscle?

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Summary. A crude extract of the sciatic nerve, eluate of dialyzed nerve extract or 2 folin-positive sephadex fractions induced acetylcholine sensitivity of extrajunctional regions in extensor digitorum longus muscle fibres of the rat, when released from silastic plates implanted for 3-4 days onto the muscle surface.

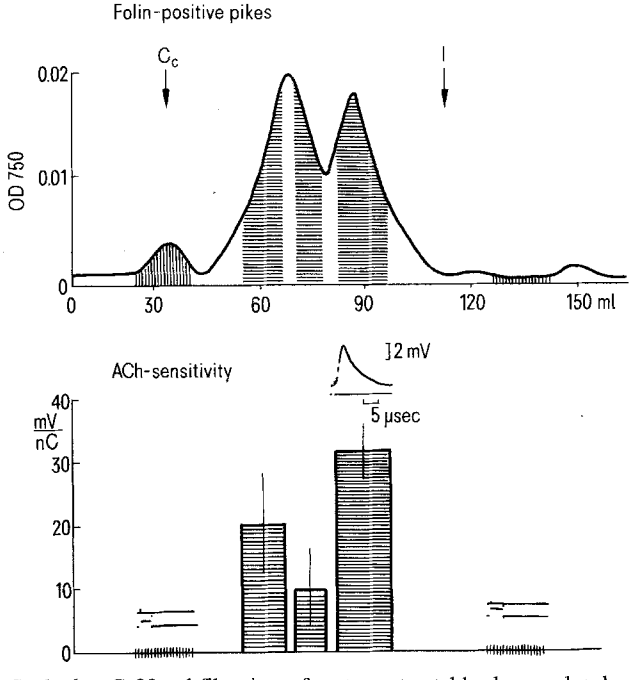
It is known that a piece of peripheral nerve placed on the extrajunctional area of skeletal muscle induces acetylcholine (ACh) sensitivity within 3-4 days[2-4]. It was suggested on the basis of these results (for review, see Gordon et al.[5]) that the nerve may contain some chemical factor which induces the formation of ACh receptors in the end plate free region of a normally innervated muscle. If this is the case, then it could be expected that ACh sensitivity will also be induced after local application of a nerve extract or its components onto the surface of muscle fibres. This assumption was tested in the present experiments on the extensor digitorum longus muscle (EDL) which was in contact with a small silastic plate containing either a crude extract of a mixed peripheral nerve, or some fractions obtained after dialysis or sephadex fractionation of the nerve.

Female albino rats (Wistar) of 180-200 g b.wt were used. Pieces of sciatic nerves (about 35 mg each) were excised from the left hind limbs, homogenized, centrifuged and the supernatant was dialyzed exhaustively for 24 h at +2 °C against water. The fraction remaining in the dialysis sac (Visking tube 8/32) and the eluate were dried under vacuum on glass dishes. Remnants of both fractions were then stirred with 20-30 mg of Silastoseal B silicon paste (Midland Silicones, England) and this mixture was transferred into polyethylene troughs and allowed to polymerize. After 24 h, when the smooth, elastic concave silicon plates (3 × 12 mm of surface area) containing the nerve fractions had polymerized, they were removed from the polyethylene forms and placed on the outer surface of the right EDL muscle, which was exposed in anaesthetized rats by dissecting the border between flexor and extensor muscles. The plates were fixed in position by sewing the tibialis anterior muscle to adjacent fascia. After 3-4 days, the EDL muscles were excised, transferred into a perfusion chamber with Liley's solution[6], and the sensitivity to ACh of the end plate free region of superficial muscle fibres was tested by intracellular microelectrodes and iontophoretic microapplication of ACh (for details of electrophysiological technique see Jones and Vyskočil[3]). The whole isolation procedure and surgery were performed under aseptic conditions.

It was found that silastic plates each containing the supernatant of crude homogenate from one piece of sciatic nerve, induced increased sensitivity to ACh in 84% of muscle fibres in end plate free parts of EDL within 3-4 days (7.3±2.2 mV/nC)[7] (mean±SE, 75 fibres, 3 muscles). Dried eluate of the dialyzed nerve extract (mol.wt under 10,000) also induced ACh sensitivity (9.2±3.5 mV/nC 88 fibres, 5 muscles), whereas the fraction remaining in the dialysis sac was ineffective (0 mV/nC in 38 fibres, only 2 fibres in 3 muscles with a sensitivity of 1.5 and 0.2 mV/nC, respectively). No extrajunctional sensitivity could be induced in control experiments by silastic plates only (3 muscles), or by plates (20 mg) containing NaCl (3.7 mg) (3 muscles), and very low sensitivity (about 0.3 mV/nC) appeared when the silastic plates contained 0.1% w/w of trypsin (30 fibres, 3 muscles).

The low molecular fraction obtained after dialysis was tested for peptides and was further fractionated on a sephadex G-25 column (1.6 × 75 cm). Peptides were eluated with 160 ml of water at a flow rate of 12 ml/h and fractions of 3 ml were collected. After reaction with the folin reagent, the eluted fractions were pooled, dried and tested for biological activity as described above. In the figure (upper part) it may be seen that 2 folin-positive peaks with mol.wts of approximately 10,000 and 8,000 were obtained. Both fractions induced ACh sensitivity when incorporated into the plates (figure, lower part). Irradiation of the dried eluate with UV-light (30 W germicide lamp at a distance of 50 cm) for 10 min abolished the sensitivity-inducing potency completely. It is known that UV-light destroys aromatic amino acids and cystein in protein molecules[8,9].

From these data, it may be concluded that the nerve contains some low molecular peptidic components which can mimic the effect of the whole nerve extract in inducing ACh sensitivity of the extrajunctional parts of skeletal muscle fibres. The results obtained by this simple procedure, however, raise several questions: What is the composition of peptides which induce ACh sensitivity, and are the peptides found in the 2 peaks after sephadex chromatogra-



Sephadex G-25 gel-filtration of water extractable, low molecular portion of the sciatic nerve (upper part) and corresponding ACh sensitivity of end plate free zone of EDL muscle. Hatched areas in the upper part of the figure represent fractions which were tested for their potency to evoke ACh-sensitivity (lower part). Abscissa in upper part: ml of eluate. Ordinate in the upper part: OD of folin reaction[13]; in the lower part: ACh sensitivity (mean±SE) expressed as millivolts of depolarization caused by 1 nanocoulomb (mV/nC)[7]. ACh sensitivity (examples of oscilloscopic records are given in insets) was tested on 250 fibres of 14 EDL muscles (2-3 muscles were tested in each case). The relative position of the standards of known mol.wts, namely cytochrome C (C_c) (mol.wt 13,000) and insulin (I) (mol.wt 6000) is indicated by arrows.

phy chemically related? What is the mechanism of ACh sensitivity induction - is it due to the increased activity of phagocytic cells[10] invading the region of EDL muscle where the proteins are released from plates, or is there a direct effect on the muscle fibre membrane?

The present results are in good accordance with the recent observation on muscle tissue cultures by Oh[11,12] who reports on a neurotrophic factor (probably a glycoprotein), capable of substituting for innervation and extractable from nerve tissue.

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Free amino acids in motor cortex of amyotrophic lateral sclerosis[1]

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Summary. Free amino acids were estimated quantitatively in the motor cortex from 3 patients with amyotrophic lateral sclerosis (ALS) and 11 control subjects. Among 7 amino acids which showed statistically significant changes, taurine was the only one which was increased constantly and most markedly in the motor cortex of all the 3 ALS cases. It was suggested that the metabolism of sulfur amino acids might be affected in comparatively early stages of ALS.

The pathogenesis of amyotrophic lateral sclerosis (ALS) is unknown. Although biochemical changes reported so far in nervous tissue of ALS might be mostly secondary phenomena, approaches along this line seem to be needed in order to find essential metabolic derangements of ALS.

Diverse changes in free amino acids in the ALS spinal cord[4] prompted us to examine the motor cortex in which histopathological changes were milder, in a sense, earlier, when compared to those seen in the spinal cord. It was found that the increase in taurine content which had been observed in the ALS spinal cord[4] was also a common change in the motor cortex of 3 ALS patients obtained at autopsy. The results of case 1 (a 52-year-old woman) have been reported previously[5].

Subjects and methods. 3 ALS patients autopsied were a 52-year-old woman, a 62-year-old woman and a 66-year-old man. They were all diagnosed as the classical, nonhereditary type of ALS by clinical and pathological findings. The total course of the illness was about 2, 1.5 and 2.5 years, respectively. The elapsed time between death and autopsy was about 3, 2 and 2.5 h, respectively. 11 control subjects had all died of nonneurological diseases, such as congestive heart failure, pneumonia, myocardial infarction, gastric ulcer, uterine myoma and neoplasms of various organs excluding the brain and the spinal cord. The age of the control subjects was 31-65 years with an average of 55. They were 5 men and 6 women. The elapsed time between death and autopsy was 2-6 h with the average of 3 h.

Precentral gray and white matters (Brodmann's area 4) weighing about 2 g were obtained and kept in $-80\,^{\circ}C$ until analyzed. Histopathology of the ALS motor cortex revealed a remarkable reduction in the number of Betz cells as well as other pyramidal cells, but there was neither marked gliosis nor demyelination. These changes in the motor cortex were obviously milder than those observed in the spinal cord[4].

Tissue was homogenized in ice-cold 1% picric acid and after centrifugation the supernatant was passed through a column of Dowex 2-X8 resin to remove picric acid. After freeze-drying the eluate, the final volume was adjusted by using a lithium citrate buffer of pH 2.15 to contain 30-200 mg of wet wt of tissue per ml. 1 ml of the final sample was applied to a JLC-6AH amino acid analyzer. Lithium citrate buffers were used in the analysis of neutral and acidic amino acids for the separation of glutamine and asparagine[6]. The value of each amino acid was expressed as μmoles per g wet wt of tissue.

Results. Among 28 free amino acids and related compounds quantitated in the ALS motor cortex, statistically significant changes were found in only 7 amino acids. Namely in the gray matter of the ALS precentral gyrus the contents of taurine, valine, leucine, isoleucine, phosphoserine and asparagine were increased. In the white matter of the same area, the contents of taurine, leucine, isoleucine and ornithine were increased. Among the amino acids changed, taurine was the only one which was increased without exception in the motor cortex of all the 3 ALS cases and the degree of its increase was most remarkable (tables 1 and 2).

Discussion. Histopathological changes in the motor cortex of the ALS patients were milder, as compared with those in the spinal cord. In a sense, the stage of the illness in the ALS motor cortex could be considered to be earlier than that in the spinal cord, where loss of nerve cells and gliosis were advanced. Actually only 7 amino acids were changed in the ALS motor cortex, while 16 amino acids were changed in the spinal cord[4]. Among these, taurine was the only amino acid which showed a common change in both the motor cortex of all the 3 ALS cases (tables 1 and 2) and the spinal cord[4]. In the spinal cord, taurine content was elevated rather diffusely with no restriction to the anterior column, anterior and lateral funiculi where histopathological changes were severe[4]. The same tendency was observed in the motor cortex where the increase in taurine content was common to both gray and white matters (tables 1 and 2). The branched-chain amino acids were increased in the ALS motor cortex (tables 1 and 2), but not at all in the ALS spinal cord[4], suggesting that these changes would not