Neuromuscular Junction Defect in Diabetes

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SUMMARY

Neuromuscular transmission has been studied electromyographically in thirty diabetic patients. The median nerve was stimulated supramaximally at the wrist, and the evoked electrical response of the abductor pollicis brevis was recorded on a cathode ray oscilloscope. Pulse trains of twenty to twenty-four stimuli were used in frequency varying from 2 to 500 per second.

In all patients the twitch rate of nerve stimulation was well maintained. Tetanic stimulation, however, was poorly sustained. A progressive decrement of the action potential amplitude occurred rapidly and earlier in many patients at frequencies that facilitated or left unchanged the amplitude in normal subjects. The drop in amplitude was particularly evident with trains of 50 and 100 per second.

The easy fatigability of neuromuscular transmission was present in clinically not involved nerve and muscle and could represent an early and only evidence of neuronal failure in diabetic patients. DIABETES 22:719-23, September, 1973.

Involvement of the neuromuscular system in diabetes has attracted considerable interest in recent years.[1] Affections of the peripheral nerves in particular, as a complication or a concomitant of the diabetic disorder, have received increased attention from clinical,[2] pathological,[3] biochemical[4] and electrophysiological[5] points of view. Measurements of motor and sensory conduction velocity have frequently indicated that a reduction in the speed of impulse propagation can be regarded as a relatively constant functional disturbance in diabetic neuropathy. Decrease in nerve conduction velocity has been demonstrated in patients in whom clinical signs of neuropathy have not yet appeared.[5,6] Of great interest are the morphologic abnormalities of the terminal neuromuscular apparatus reported in patients with diabetes but without overt signs of neuropathy.[7] It can be helpful, in evaluating the peripheral nerves and muscles in patients with diabetes, to investigate not only the membrane phenomena using EMG and nerve conduction but also the characteristics of the neuromuscular transmission. The process is conveniently studied by recording the behavior of the muscle summated electrical response for repetitive stimulation of its peripheral nerve.

Failure of neuromuscular transmission was evident in many diabetic patients in the present study, suggesting a disturbance of those complex metabolic mechanisms mediating normal neuromuscular activity.

MATERIAL AND METHOD

Thirty diabetic patients were studied together with twenty normal subjects without signs or symptoms of neuromuscular disease. The range of age in the diabetic group was forty-one to sixty-eight years (mean age fifty-two) and in the normal group twenty-two to sixty years (mean age forty).

Diabetes was controlled either by diet alone or with oral medication, and its duration varied from three to twenty years with an average of nine years. All patients were selected because they had one or more signs of peripheral neuropathy at clinical or electromyographic examination; however, the conduction velocity of the motor and sensory fibers of the median nerve tested was within normal limits. Unusual fatigability of neuromuscular transmission is detected by repetitive supramaximal stimulation of a peripheral nerve. In recording the muscle action potential as a measure of its response, a decline in the amplitude of the evoked potentials will be observed as individual muscle fibers fail progressively to respond when the nerve is stimulated at frequencies which a normal muscle could endure for a long period of time.

The median nerve was stimulated with a bipolar stimulator strapped at the wrist. Pulse durations of 0.1 msec. were used, and the intensity increased to give a supramaximal stimulus. The stimulator had a ground isolated output (Teca S1-3) and the stimulus output,

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under load conditions, was verified to be uniform from pulse to pulse, including the start of the pulse train. Trains of twenty to twenty-four stimuli were delivered at frequencies of 2, 5, 10, 20, 50, 100, 200 and 500 per second. A rest period of two to three minutes was allowed between consecutive trains.

The evoked action potentials, negative deflection up, were recorded with surface electrodes placed over the abductor pollicis brevis in the usual belly-tendon position. Measurements of the action potential peak-to-peak amplitude were made from tracings displayed on a Tektronix storage scope (model 564) and from photographs.

The median nerve sensory action potentials were recorded antidromically in five normal and ten diabetic patients. The nerve was stimulated at the wrist and the pickup electrodes placed along the index finger. The frequencies used were the same as those used for motor stimulation. The arm and the hand were firmly fixed in place on a molded plaster armboard which prevented gross movements.

RESULTS

Normal subjects. The peak-to-peak amplitude of the evoked electrical response remained essentially unchanged with repetitive stimuli at slow rates (2 to 5 per second). A progressive increment of the response was observed with pulse trains of 10, 20, 50 and 100 per second (figure 2). The amplitude generally increased during the first eight to ten pulses and showed little or no further changes. The highest increment was observed with trains of 20 per second. Comparing the amplitude of the twentieth response with the first one in each train, it can be seen from figure 2 that the amplitude increased by an average of 17 per cent at 10 per second stimulation, by 30 per cent at 20 per second, by 18 per cent at 50 per second and by 9 per cent at 100 per second stimulation. Frequency of 200 per second reduced the amplitude of the evoked electrical response in all subjects by an average of 57 per cent. Stimulation at 500 per second was ineffective and no responses could be observed after the first one at that high frequency.

Patients. A rapid and early fatigability of neuromuscular transmission was evident in most of the patients. A decrement in the amplitude of the evoked electrical response was evident with a train of stimuli which was facilitatory in normal subjects (figures 1 and 2). Frequencies of 10 per second provoked a slight decrement of the response in six patients, though the effect generally was facilitatory with an average increase of 12

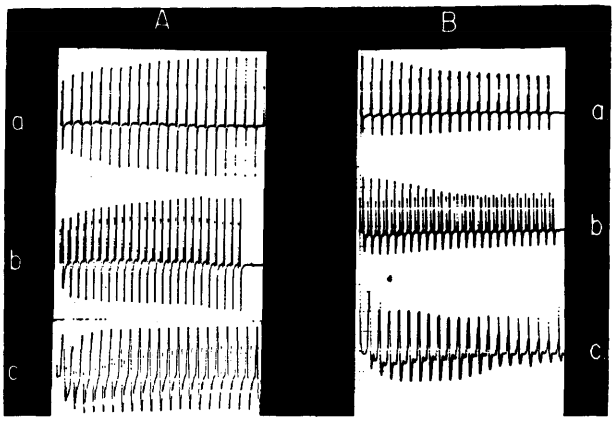

FIG. 1. Changes in the action potential amplitude of the abductor pollicis brevis on supramaximal stimulation of the median nerve at the wrist. Response to short train of stimuli at frequency of (a) 20 per second, (b) 50 per second and (c) 100 per second. Note increment of successive responses in normal subjects (A) and progressive decline in diabetic patients (B).

per cent. At 20 per second stimulation the average increment was only 3 per cent and in thirteen patients the amplitude decreased 10 to 35 per cent. At 50 per second stimulation the amplitude dropped by an average of 10 per cent. At this frequency an increment, ranging from 5 to 35 per cent, was present in only ten patients, and the amplitude either decreased or remained

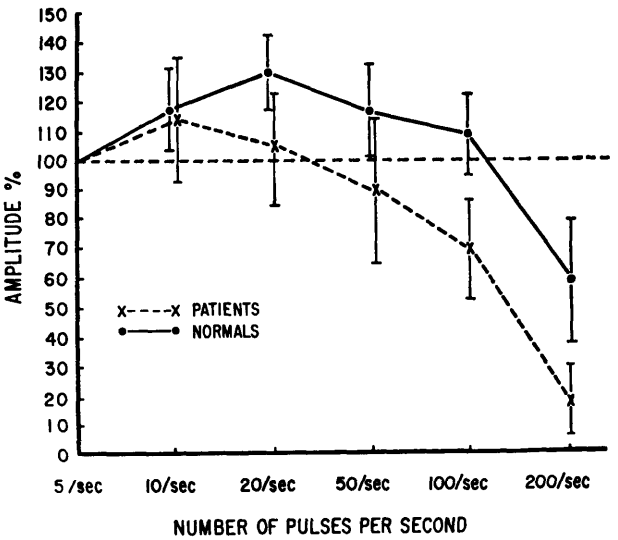

FIG. 2. Mean amplitude of the abductor pollicis brevis electrical response evoked by short train (twenty pulses) of supramaximal stimuli of the median nerve at the wrist. Abscissa: frequency of stimulation. Ordinate: peak-to-peak amplitude of the twentieth response as a percentage of the first response of each train. The vertical bars denote standard deviation. Mean values in twenty normal subjects and thirty diabetic patients.

the same in the remaining twenty patients. Trains of 100 per second caused a rapid fall of the potential in all patients; the response dropped by an average of 30 per cent. The exhaustion of the response at 200 per second stimulation was most rapid and severe, decreasing to zero or a trace in many of the patients. The average decrement was 83 per cent. Results are summarized in figure 2. No attempt was made in the present study to correlate the duration and/or severity of diabetes with the behavior of the evoked electrical response. Patients were selected on the basis of their well known history and findings of diabetes and, as stated above, diabetes was controlled either by diet alone or with oral medication.

The decrement of the muscle electrical response was not qualitatively different from that seen in myasthenia gravis. In the latter condition, however, the decrease of the response usually occurs with pulse trains of low frequency (3 to 5 per second) as well as high frequency (10 to 50 per second), while in most of our patients the response remained unchanged with low frequencies and decreased only with relatively high rates of stimulation (50 to 100 per second).

DISCUSSION

Failure of neuromuscular transmission is classically associated with myasthenia gravis but not exclusively with this condition. Apart from the not infrequent association of myasthenia with such disorders as systemic lupus erythematosus, polymyositis, dermatomyositis and hyperthyroidism,[8] myasthenic-like responses have been described with poliomyelitis,[9] amyotrophic lateral sclerosis,[10] syringomyelia[11] and in rare cases of peripheral neuropathy.[12] The significant* decrement of the evoked electrical response in our patients, with brief pulse trains of rapid stimuli well sustained by normal subjects, points to an easy fatigability and suggests that a neuromuscular transmission defect accompanies diabetes. The mean age of the patient group was greater than that of the normal subjects, and age is known to influence such parameters as nerve conduction velocity, duration of action potentials and number of complex potentials. However, findings are quite consistent in the adult population and only minor statistical differences, still within the limits of normal, can be found between young and elderly subjects. As reported in the results, a decrement of the response started to appear in some patients at a frequency of stimulation of 10 per second and became more dramatic with 20 and 50 per second trains. No such decrement was observed in the normal subjects, even in those who were older than some of the patients used in the study.

Neuromuscular transmission occurs in a complex series of electrical and biochemical steps, and failure may occur at a number of different sites or ways. It is reasonable to assume that the depression observed in our patients is the result of a disturbance in the normal mechanism of transmission along and across the neuromuscular junction. The direct recording of the sensory action potential along the digital fibers of the median nerve in the finger failed to show any decrement of the response, even with frequency of stimulation of 200 per second, which, as shown, produced a severe decline of the muscle action potentials in normals and patients alike. Similar observations have been reported by Alajouanine et al. in myasthenia gravis.[13]

The decrement observed in the diabetic patients at frequencies of 20 and 50 per second is particularly significant since these are the frequencies with which motor units are usually activated during maximal voluntary contraction. It could be that the early and frequent complaints of weakness and fatigability of many patients are due to the initial failure of the neuromuscular apparatus in diabetes. Normal motor function depends upon, among other things, the functional and anatomic integrity of the neuromuscular apparatus, and recent biochemical and histologic reports[7,14] have focused attention on the changes and defects that may occur in diabetes.

In the complex metabolic processes taking place at the synaptic level and involving the metabolism of the essential chemical transmitter acetylcholine, an important role is played by glucose as energy source entering the citric acid cycle with production of ATP.[15] The synthesis of acetylcholine by choline acetylase is dependent on the continued supply of choline and of glucose.[16] Poor glucose utilization, therefore, could indirectly affect the function of the neuromuscular transmission. On the other hand, a specific defect of acetylcholine formation, release or resynthesis cannot be excluded. Interesting in this respect are the findings of Feldberg,[17] who demonstrated that elevated concentrations of glucose of 200 mg./100 ml. or higher inhibit the synthesis of acetylcholine by nerve tissue.

The nerve terminal after losing its myelin, at the end plate region, becomes shrouded by the Schwann cell, which thereby separates the axon terminal from

*The t test was used (two tails) and the difference between normal subjects and patients at 50, 100, and 200 per second stimulation was significant at 0.005 level.

the surrounding tissue. There is a widespread and growing feeling that the Schwann cells play an active part in the function of the synaptic region.[18] Recent electronmicroscopic and biochemical findings show an unequivocal relationship between the underlying diabetes and the metabolic defects in the Schwann cell.[14,19,20] This cell undergoes considerable change in diabetes. Disturbances in intracellular lipid metabolism have been shown in addition to those in intracellular carbohydrate metabolism.[14] Intracellular glucose as well as fructose have been found greatly increased in diabetic preparations, the latter apparently due to a direct conversion of glucose to sorbitol by the action of aldolase reductase and its subsequent oxidation to fructose ("sorbitol pathway").[20] The accumulation of fructose which cannot be further utilized has been regarded as having a fatal osmotic effect.

This biochemical view of diabetic neuropathy as a metabolic disease affecting Schwann cell integrity is supported by the predominant histologic findings of segmental demyelination in human diabetic neuropathy.[21]

With respect to the neuromuscular junction, histopathologic changes have been described involving primarily the axon terminal arborization.[22] Woolf and Malins have reported characteristic degenerative changes confined to the motor end plates in diabetes.[23] The degenerative changes have been demonstrated even at a time when the proximal nerve fibers were unaffected, suggesting that the disease may attack the end plate primarily.[24] This view has received support from the recent studies of Coërs and Hildebrandt,[7] who studied diabetes at various stages by several electrophysiologic and histologic means. The first morphologic indication of neuronal involvement constantly observed was limited to the level of the neuromuscular junction as a reduction, irregularity or abnormal expansion of motor end plates. These changes, which had no clinical expression, were associated neither with histologic changes in muscle tissue nor with abnormal electromyographic patterns.

Reske-Nielsen et al. have shown well marked abnormalities of the terminal neuromuscular apparatus in recent juvenile and long-term diabetes.[25] They demonstrated that severe abnormalities were present at the start of the disease.

The fatigability of neuromuscular transmission reported in the present study could indeed be a reflection of the metabolic and/or morphologic changes taking place early in the development of diabetes. It must be pointed out that the conduction velocity and the distal latency of the median nerve were within normal limits in all patients. The failure of neuromuscular transmission could, therefore, constitute the sole evidence, in the absence of other abnormalities, of incipient neuronal failure.

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BOOK REVIEW

ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, VOLUME 26: PHARMACOLOGICAL CONTROL OF LIPID METABOLISM, *edited by William L. Holmes, Rodolfo Paoletti, and David Kritchevsky, $22.50, 359 pages. New York, Plenum Press, 1972.*

This volume represents one of a series on a variety of subjects which are currently under intensive investigation in the biomedical community including topics in cell biology, endocrinology, hematology, and atherosclerosis. The book contains the texts of invited papers and reproductions of abstracts submitted to the Fourth International Symposium on Drugs Affecting Lipid Metabolism, which was held in Philadelphia, September 8-11, 1971. The status of knowledge is beautifully reviewed by the "leading lights" in lipid and lipoprotein chemistry and lipid and lipoprotein pharmacology and in heart disease prevention. Among the areas covered are: synthesis and secretion of lipoproteins, apolipoproteins, mechanisms of hyperlipoproteinemia, lipoprotein lipase, effect of drugs on triglyceride metabolism and treatment of hypercholesterolemia. The principal investigators of some of the leading primary and secondary coronary prevention trials review their experiences. Thus, here one can find a great deal of the basic information on which the current biochemical and epidemiological concepts of coronary prevention are based. The inclusion of the abstracts gives one a preview of "coming attractions." The material is suitable for those with a modern biochemical and medical background who have had some interest in this area.

There is one glaring omission. Some of the invited speakers submitted very abbreviated abstracts of their presentations; this is unfortunate. Presumably not all readers will be thoroughly knowledgeable in the field; to this kind of reader, the utility of the book is diminished by these omissions. One further criticism of the book is its format and hence its cost. Since these are highly technical volumes, the materials in them have a rather short "half-life." Furthermore, similar reviews appear elsewhere. Therefore, although all of us enjoy seeing our words carved in granite, a paperback volume costing much less would have served equally well.