

Title: A PREJUNCTIONAL EFFECT OF NEOSTIGMINE ON THE TERMINALS OF PHASIC AND TONIC MOTOR NERVES IN ADULT AND AGED RATS

Authors: T. Baker, M.S. and A. Stanec, M.D.

Affiliation: Department of Pharmacology, Cornell University Medical College, New York, New York 10021 and Department of Anesthesiology, St. Joseph's Hospital and Medical Center, Paterson, New Jersey 07503

Introduction: The differences between the phasic and tonic muscle are well established. Among these are speed of contraction, relaxation time, the amount of tension developed in response to neural stimulation and fatigability. Many of these phenomena are dependent upon neural trophic factors since after cross-innervation, each type of muscle will begin to develop some of the characteristics of the opposite muscle type. Electrophysiologic differences between tonic and phasic motoneurons have also been well defined: soma size, axonal conduction velocity; input resistances, range of the dendritic electronic length, durations of the after-hyperpolarization and membrane time constants. The experiments reported herein were undertaken to investigate, first, differences in the terminals of motor nerves innervating phasic and tonic muscle and secondly, how these differences are affected by age.

Methods: The differences between the terminals of phasic and tonic motor nerves were studied pharmacologically using neostigmine which acts directly on motor nerve terminals by conditioning these terminals to generate repetitive afterdischarges evoked by stimulation of the nerve.¹ Although this drug is an anticholinesterase, Riker has suggested that agents like neostigmine should be known as facilitatory drugs since they facilitate function in the neuromuscular junction.² The neural stimulus-bound repetition, known as SBR, generated in the motor nerve terminals by facilitatory agents causes an obligatory potentiation of the muscle contractile response, called post-drug potentiation (PDP). Thus, the neural SBR and PDP evoked by neostigmine were used as indices of motor nerve terminal function. Neostigmine was administered intraperitoneally with the various doses in a constant volume of 1 ml/Kg with each rat receiving only one dose of neostigmine. Two groups of Sprague Dawley rats were used: adult males weighing 350-450 g (12 to 14 weeks old) and aged male rats (24 to 27 months old) weighing 550-800 g. Both groups were anesthetized intraperitoneally with a 1000 mg/Kg dose of urethane. A second dose, 400 mg/Kg was given subcutaneously after the rats became unconscious. The duration of the anesthesia was 6 to 8 hours. The rats were tracheostomized and were artificially ventilated with air at PaCO₂ of 35-40 torr. In one leg a soleus (tonic) nerve-muscle preparation and in the contralateral leg a medial gastrocnemius (phasic) nerve-muscle preparation were employed. In every other rat, the type of nerve-muscle preparation in the right and left legs was rotated. The stimuli were supramaximal rectangular pulses of 0.1 msec duration delivered once every 5 seconds (0.2 Hz). In those experiments which SBR was recorded, a dorsal laminectomy was performed from L1 through S1. Ventral roots L4, L5 and L6 were cut proximal to the spinal cord, and those roots containing axons innervating the muscles under study were identified. From these roots small filaments containing 1 to 3

axons that innervated the medial gastrocnemius or soleus muscle were placed on recording electrodes and SBR activity was recorded.

Results: In young adult rats, neostigmine was twice as potent in causing SBR in the terminals of motor nerves innervating the medial gastrocnemius muscle than in soleus motor nerve terminals. The duration of the SBR in the soleus motor nerve terminals, however, was significantly longer ($p < 0.05$) than that of the gastrocnemius SBR. Dose response regressions of the PDP also showed that the gastrocnemius muscle was twice as sensitive to neostigmine than the soleus muscle. The intensity and duration of the SBR activity correlated well with the amount and duration of PDP. In the aged rats, the PDP response of the two muscle were similar in peak effect and duration. The peak effect was only one-third of that in the young adult rats and the duration was longer. The incidence of SBR was less while its duration in those axons demonstrating SBR was longer. Again SBR activity and duration correlated well with the intensity and duration of PDP. Data from single axon recordings suggest that in young adult rats, the frequency of the gastrocnemius SBR is higher than that of the soleus SBR. The number of spikes in the SBR train, however, is greater in soleus than in the gastrocnemius. In the aged rat, these differences do not appear to exist.

Discussion: Although major differences in the electrophysiology between phasic and tonic motoneurons have been long known as well as those in the prejunctional ultrastructure of these two nerve types, pharmacologic differences between them have not been readily understood or considered to have clinical significance. The data of this report show that drug effects on the terminals of these two types of motor nerve can account for some of these pharmacologic differences. Since most human muscles have varying ratios of phasic and tonic motor units, it is not surprising that the pharmacologic responses of different muscles to facilitatory drugs vary widely. This fact reflects that prejunctional sites of facilitatory drug action play a much more important role in the clinical actions of these drugs than previously thought.

References:

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