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Measurement of Fasciculations as Motor Nerve Ending Discharges in the Rat: A Dose Related Effect of Neostigmine[1] (42014)

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Abstract. The muscle fasciculations caused by neostigmine and similar agents are the result of a primary drug action on motor nerve endings. Asynchronous, repetitive firing of action potentials are evoked at motor nerve endings which are then transmitted to muscle. A dose–response relationship between neostigmine dose and the rate of/or total neural activity has been established in the rat. This fasciculatory response to neostigmine can serve as an index of motor nerve ending excitability and may be useful in assessing the effects of certain pathological states or drug actions at the neuromuscular junction. © 1985 Society for Experimental Biology and Medicine.

The occurrence of fasciculations follow-the administration of anticholinesterase agents, such as neostigmine or physostigmine, and depolarizing drugs, such as succinylcholine or acetylcholine, has been long recognized [cf. (1, 2)]. These drug-induced fascicular contractions originate at motor nerve endings (3–10) and appear as single or repetitive neural events which are then transmitted to muscle. In this context, motor nerve endings are that portion of peripheral motor axons extending from the distal or last node of Ranvier and include the unmyelinated terminal. Although the phenomenon is fairly well known, no dose–response relationship between these drugs and fasciculations has been demonstrated. The results of the present investigation show that such a relationship does exist for neostigmine.

Methods. Since fasciculations originate on the motor axon, action potentials of opposite direction are propagated from this locus. The othrodromic action potentials result in neuromuscular transmission and evoke fascicular muscle contractions. The antidromic action potentials travel up the axon into the ventral root. Monitoring of these neural discharges was accomplished *in situ* by recording the antidromic action potentials from rat ventral roots L5 and L6. This approach was superior to alternative means of detection (e.g., muscle tension recordings or electromyography) in that the ventral root recording provided the most direct readout of events occurring in the motor nerve endings.

Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, Mass.) weighing 275–350 g were anesthetized with urethane given intraperitoneally as a 40% solution in a dose of 0.2 ml/100 g of body weight and later supplemented subcutaneously with 0.2 ml/100 g. A dorsal laminectomy from L1–S1 was performed and the dura mater of the exposed spinal cord was opened. The animal was mounted in a rigid metal frame and the skin edges elevated to form a basin which was filled with paraffin oil maintained by radiant heat at 37°C. Ventral roots L4, L5, and L6 were teased away from the cauda equina by means of long slender glass hooks and sectioned centrally close to the cord. The ventral root selected for study (L5 or L6) was then placed on a bipolar platinum recording electrode.

Action potentials detected in the ventral roots were amplified by differential amplifiers and power supply (Tektronix PS 501-1/PS 502). The output was connected in parallel to a Nicolet 1072 window discriminator–histogram analyzer and a Tektronix 2A63/

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565 dual beam oscilloscope. All neural discharges above a baseline threshold were counted by the Nicolet 1072 regardless of their final amplitude. The total number of action potentials observed, their rate of occurrence (sec^{-1}), the time to peak rate, duration, and half-time were calculated. A Vetter Model B tape recorder preserved each experiment for later review. Photographs of the oscilloscope tracings were made with a Grass C4 Kymograph Camera on Kodak RAR film 2495.

Neostigmine was injected via a cannula placed in the right jugular vein. The solution concentration was adjusted so that the desired dose per kilogram was administered in 1.0 ml saline. Each rat received only a single dose of neostigmine, and the response to each dose studied was determined in four or eight animals. In each series of rats used to study the effects of a particular dose, action potentials were recorded in half of the animals from L5 and in the remaining half from L6.

Neostigmine bromide and ethyl carbamate (urethane) were purchased from Sigma Chemical Company of St. Louis, Missouri.

Results. Figure 1 shows the asynchronous neural discharges which were induced by neostigmine and which evoked muscular fasciculations. These tracings were recorded from ventral root L5 of a rat which had received intravenously a neostigmine dose of 12.5 μg/kg, 5.5 min earlier; at this time in the experiment, neural activity was at peak. Following the neostigmine injection, first singles and then pairs of spontaneous action potentials were recorded. At peak activity, trains of three or more repetitive action potentials were regularly observed. As the activity waned, trains of three or more occurred less frequently. With time, only single and occasional doublets of action potentials occurred until all activity stopped.

The intensity of the fasciculatory response directly increased with the neostigmine dose. This was demonstrated by counting the neural activity at peak (Fig. 2A) or by measuring the cumulative neural activity during the entire experiment (Fig. 2B). Neostigmine doses less than 6.25 μg/kg iv did not regularly produce fasciculations or spontaneous neural action potentials. Doses larger than 25 μg/kg iv resulted in action potentials too numerous to precisely quantitate.

Figure 3 shows the time course of neural activity evoked in eight rats by a neostigmine dose of 12.5 μg/kg iv. Peak neural activity was quickly reached and briefly maintained; neural discharges then declined in frequency until at 40 min after the administration of neostigmine all neural activity stopped. The time to peak activity, the total duration of the asynchronous neural activity and the functional half-time (i.e., the time elapsed from peak activity to one-half peak) as a function of neostigmine dose are shown in Table I. Not suprisingly, as the dose of neostigmine was increased the time to peak decreased. The duration of neural activity and the half-time of peak decay, however, were directly proportional to the dose size.

Although not quantified in this study, the time courses and intensities of the muscular fasciculations evoked by the neostigmine doses were similar to those reported here for neural discharges. Electromyographic recordings of muscle fasciculations have been made simultaneously from the rat biceps femoris while recording neural discharges in ventral roots L5 and L6, which contain the motor axons innervating the biceps femoris (11). High degrees of correlation were found between both the peak rates of muscle fasciculations and neural discharges ($r = 0.92$), and the total accumulative numbers of muscle fasciculations and neural discharges ($r = 0.95$).

Discussion. Hall *et al.* (12) proposed that neostigmine-induced fasciculations result from an initial random depolarization of unmyelinated motor nerve terminals. If this depolarization is sufficiently large, an action potential develops at the distal Ranvier node as a consequence of cathodal current flow from this region to the motor nerve terminals. Repetitive firing ensues if this current flow and the potential differences responsible for it are maintained and if the excitation threshold of the distal node is exceeded for a sufficient time. Transmission to muscle depends upon these neural events and results in the asynchronous contraction of individual motor units, i.e., fasciculations.

The increase in intensity of the neural activity with neostigmine dose observed in the present experiments reflects both an increase in the total number of action potentials evoked at each motor nerve ending and an

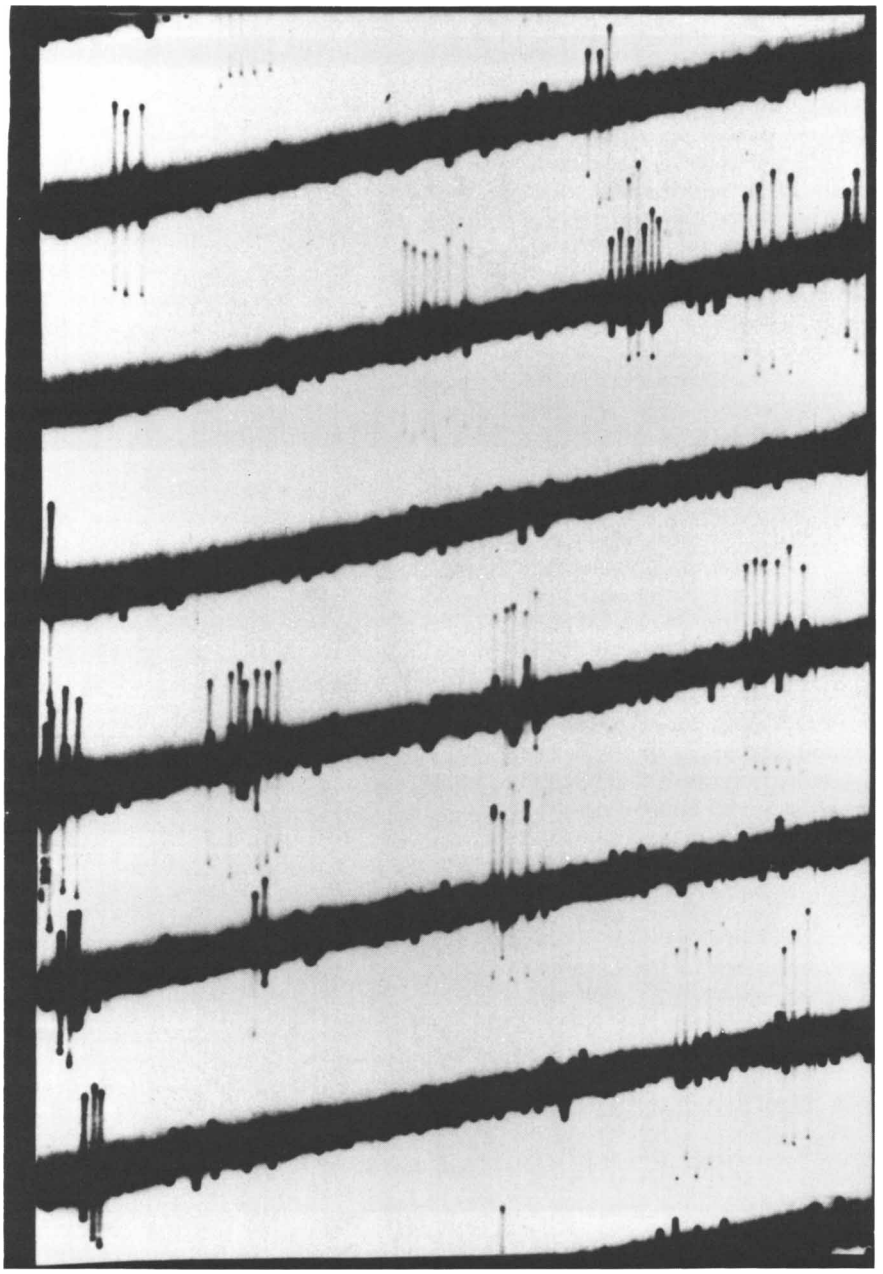

FIG. 1. Asynchronous neural discharges induced by neostigmine 12.5 μg/kg iv given 5.5 min earlier. Neural activity is at peak. Action potentials are shown as positive and negative deflections from the baseline. Each sweep represents 350 msec.

increase in the proportion of motor nerve endings actively firing at a given time. The decay in intensity of neural activity probably is the result of neostigmine elimination from the active sites. Elimination could be via redistribution, filtration and secretion by the kidneys, and biotransformation. Drug-induced transmission block is unlikely to be responsible for this decay as the peak response, the duration of drug effect and the half-time were increased directly with neostigmine dose (Fig. 3 and Table I).

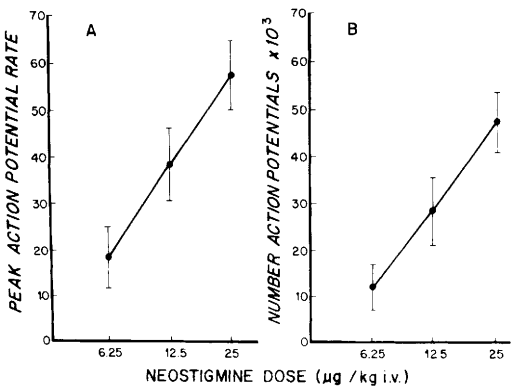

FIG. 2. Dose–response curves for neostigmine-induced neural discharges. (A) Peak rate of action potentials firing (sec^{-1}). (B) Total number of action potentials which occurred during experiment. Bars represent ± SEM, where n = 8, neostigmine dose = 6.25 or 12.5 μg/kg; where n = 4, neostigmine dose = 25 μg/kg.

Depolarizing drugs like acetylcholine and succinylcholine also induce asynchronous firing of motor nerve endings (7, 10), probably by the same mechanism proposed by Hall and his co-workers (12). This explains the gross fasciculations evoked by succinylcholine observed in anesthetized patients. Physostigmine, neostigmine, acetycholine, and succinylcholine can directly depolarize motor end plates. Such an action, however, would cause fibrillations, not fasciculations. That fasciculations do occur (as coordinated events) indicates that a pacemaker drives the muscle fibers of a fascicle in unison. As discussed by Riker (13), the most appropriate pacemaker is the motor nerve innervating the fascicle. Specifically, these agents are either quaternary ammonium ions or exist mostly as charged moieties at body pH. As such, they have poor access to internodal regions of the axon. Therefore, these agents more readily react with the unmyelinated nodes of Ranvier, terminal axons, and terminal aborizations—the motor nerve endings.

Quantitation of neostigmine-induced fasciculations represents a useful index of motor nerve ending excitability. In this context, motor nerve ending excitability is defined in terms of two functions: (a) an inverse function of the distal node excitation threshold, and (b) a direct function of the depolarizing capacity of the motor nerve terminals. If the threshold of the distal node is increased, neostigmine-induced repetitive firing would be reduced as a result and motor nerve ending excitability may be said to be depressed. If the motor nerve terminal depolarization (and its subsequent afterpotentials) is diminished in amplitude or duration, the effect on neostigmine-induced repetitive firing and motor nerve ending excitability would be the same. Consequently, the intensity of the motor nerve ending fasciculatory response to neostigmine reflects threshold or excitability changes occurring at these minute structures. Drug pretreatment or pathologic factors which alter neostigmine-induced fasciculations do so by a direct effect on motor nerve ending excitability as suggested by studies of the actions of *d*-tubocurarine (3, 14), adrenocortical insufficiency (15), and amyotrophic lateral sclerosis (16).

The use of rats in the present experiments offers several advantages over other animal species (e.g., cats) for measuring motor nerve ending excitability: (a) Anatomically, the rat ventral roots innervating the hind limbs are contained within a lengthy cauda equina. As a result, the roots are extremely easy to isolate and manipulate; excessive bleeding from the spinal cord and "dying back" of the root from the point of sectioning are not a problem. (b) Surgically, the laminectomy in the rat is less labor intensive and less liable to mechanical error. (c) Lower animal costs and a more uniform genetic pool are also factors to be considered.

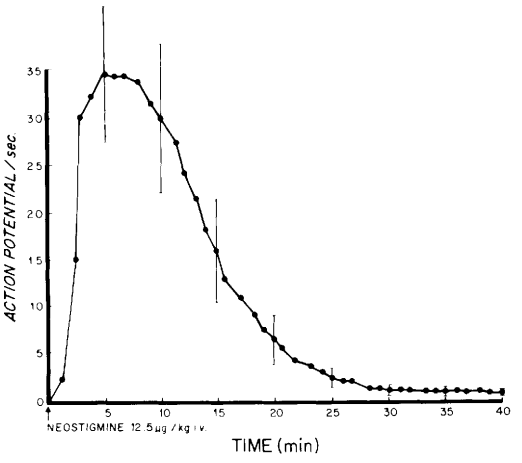

FIG. 3. The time course of mean neural discharge (action potential) activity induced by neostigmine 12.5 μg/kg iv. Bars represent ± SEM, where n = 8.

TABLE I. TIME COURSE OF NEOSTIGMINE-INDUCED NEURAL DISCHARGE (ND) AS A FUNCTION OF DOSE[a]

Neostigmine dose (μg/kg iv)	Time to peak ND rate (min)	Duration (min)	Half-time[b] (min)
6.25	6.4 ± 0.5	21.2 ± 3.3	5.4 ± 0.7
12.5	5.8 ± 0.6	27.9 ± 1.8	7.6 ± 0.5
25.0	3.2 ± 0.8	39.2 ± 2.1	10.5 ± 0.5

[a] Results are expressed as means ± SEM for $N = 4$ or 8.

[b] Half-time was calculated as the time elapsed from the peak rate to half of this value.

This method has allowed for the first time the definition of the dose–response relationship between neostigmine and fasciculations. It also provides a means of examining the effects of various agents on motor nerve excitability and should help describe structure activity relationships and the molecular basis of drug actions on motor nerve excitability.

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