Anesthesiology
V 38, No 2, Feb 1973

The Effects of Gallamine on Nerve Terminals and Endplates

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Using standard microelectrode recording techniques, the effects of gallamine on nerve terminals and endplates of the isolated frog sartorius muscle were evaluated. In low concentration (10^{-4} g/l), the agent increased MEPP frequency and twitch strength and caused antidromic firing from the nerve terminal. In the concentration range of (5×10^{-5} to 5×10^{-4} g/l), which inhibited twitch height, nerve-terminal activity remained elevated. In this range, MEPP amplitude and endplate sensitivity were markedly reduced, and resting membrane potential was reduced by 10–15 mV. At high doses (10^{-3} g/l) gallamine finally inhibited nerve-terminal function. The reduction in twitch height was explained by blockade of the postjunctional membrane. It is postulated that gallamine has three effects on the myoneural junction: 1) stimulation of the nerve terminal at low doses; 2) reduction of endplate sensitivity to acetylcholine concomitant with reduction in twitch height at higher doses; 3) inhibition of nerve-terminal function at the highest doses studied. (Key words: Gallamine; Nerve terminal; Neuromuscular transmission; Endplate.)

GALLAMINE is usually considered to have neuromuscular blocking activity similar to that of curare, in that it has a depressant action on the postjunctional membrane. This impression has been gained principally from nerve–muscle twitch tension studies.[1, 2] However, curare has pronounced effects on the nerve terminal.[3] Evidence that the neuromuscular blockade produced by curare is primarily a presynaptic phenomenon has also been cited.[4, 5] In addition, *d*-tubocurarine has a stimulatory action on the nerve terminal at concentrations far below those needed to produce blockade.[5]

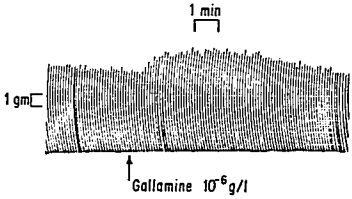

FIG. 1. Effect of Gallamine, 10^{-4} g/l, on twitch height of the indirectly-stimulated frog sartorius muscle. This record is typical of ten muscles.

The purpose of this work was to examine the effects of a wide range of concentrations of gallamine on spontaneous acetylcholine release, twitch height, endplate sensitivity to acetylcholine, and nerve-terminal activity recorded from the ventral root. In this way the relative effects of gallamine on the motor nerve terminal and endplate could be determined.

Methods

FROG SCIATIC NERVE–SARTORIUS MUSCLE TWITCH PREPARATION

Studies of the effects of gallamine (Lederle Laboratories) on the twitch strength of the indirectly-stimulated frog sciatic nerve–sartorius muscle preparation were conducted as described previously.[5]

RECORDING AMPLITUDE AND FREQUENCY OF MINIATURE ENDPLATE POTENTIALS

The methods of recording miniature endplate potential (MEPP) amplitudes and frequencies and sensitivity of the endplate region to iontophoretically applied acetylcholine were

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Received from the Departments of Anesthesia and Pharmacology, School of Medicine, University of Iowa, Iowa City, Iowa 52240. Accepted for publication September 18, 1972. Supported in part by USPHS grants NS-4431 and NS-1396 and USPHS training grant 5 T0 GM00141-15 and the Department of Anesthesia Trust Fund.

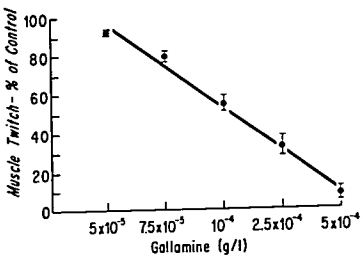

FIG. 2. Effects of gallamine, 5×10^{-5} to 5×10^{-4} g/l, on twitch height of the indirectly-stimulated frog sartorius muscle. Values are means ±SE, n = 10.

similar to those described by del Castello and Katz [6] and Sokoll *et al.*[5]

Denervation of Frog Sartorius Muscle

Frogs (*Rana pipiens*) 2 to 2½ inches long were anesthetized by immersion in a solution of 5 per cent ether in water for 2 to 3 minutes.[7] The skin of the dorsal part of the left leg was incised longitudinally for a length of about 10 mm above the thigh. The branch of the sciatic plexus which innervates the sartorius muscle was isolated and a 4–5 mm segment was removed. The skin was then closed with four to five sutures.

Groups of three frogs each were kept in plastic animal cages in shallow water at room temperature. The water was changed every other day. For the first week the frogs were fed tetracycline, 5 mg in 0.2 ml of water, twice a day by stomach tube. This treatment has been shown to be very effective in preventing and curing red-leg disease.[8] After this, they were fed one crushed mealworm every other day by stomach tube until they were sacrificed. The animals were kept in this way for five to six weeks. MEPP frequencies and amplitudes for these animals were recorded as previously described for normal frogs. In addition, sensitivity of the endplate to iontophoretically applied acetylcholine was investigated.

Nerve-terminal Recordings

Frogs (*Rana cathesbiana*) 6 inches long were placed in an environment of −15 C for 90 minutes to induce hypothermic anesthesia. A midline incision was made on the back and the spinal and transverse processes were ex-

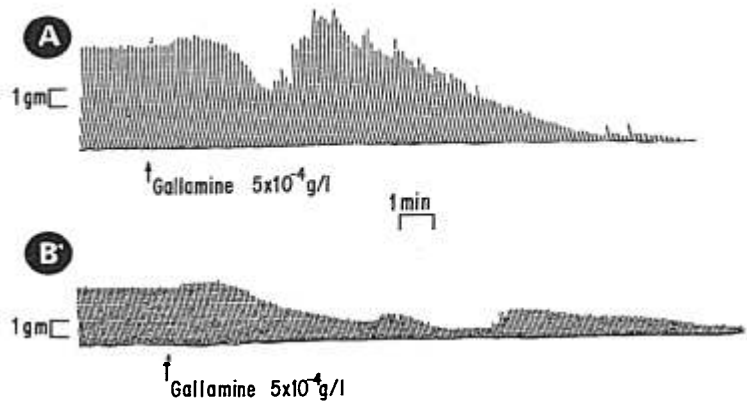

FIG. 3. Patterns of the blockade induced by gallamine, 5×10^{-4} g/l, in the indirectly-stimulated frog sartorius muscle. Arrow indicates when drug was administered. Each of these records is typical of ten muscles. *A*, onset of blockade showing one episode of relatively increased twitch height: *B*, onset of blockade showing multiple episodes of relatively increased twitch height.

posed. Laminectomies of vertebrae 4 to 9 were performed to expose the spinal cord. The cord was cut and rotated 180 degrees to expose the ventral roots. The roots of spinal nerves 7, 8, 9 and 10, which comprise the sciatic plexus, were sectioned as close as possible to the spinal cord. The sartorius muscle was freed from its attachment to the knee and a suture was placed around the tendon. This suture was connected to a Grass strain-gauge transducer, while the muscle rested in a horizontal bath filled with frog Ringer's solution. The nerve supply to the sartorius muscle was isolated and all other branches of the sciatic plexus were sectioned.

The individual ventral roots were stimulated with bipolar electrodes to determine which innervated the sartorius muscle. This particular root was pulled into a pipette containing frog Ringer's solution and a silver–silver chloride electrode. Monopolar action potentials, recorded from the ventral root and an indifferent electrode, were monitored using a Tektronix 564B storage oscilloscope. A stimulating electrode was placed on the nerve in the region of the thigh. All exposed nerves were covered with oxygenated mineral oil. The animal was warmed to room temperature before the start of the experiment. Stimulation conditions were 0.2 Hz for 0.1 msec at supramaximal voltage. Tetanus was induced by a train of pulses of 10 Hz for 5–10 seconds. All drugs to be tested were added to the bath. The various concentrations of gallamine were administered in random order to six animals.

This methodology is based on the principle that following orthodromic nerve stimulation an increase in nerve-terminal activity will be reflected by the appearance of antidromic action potentials recorded at the ventral root of the spinal nerve.[9]

The same apparatus can be used to determine whether certain drugs have inhibitory effects on the motor nerve terminal. Following a period of tetanic stimulation, the height of the muscle twitch will be increased on subsequent nerve stimulation. This is referred to as "posttetanic potentiation" (PTP). Associated with this is increased firing of the nerve terminal, which is called "posttetanic repetitive firing" (PTR). This PTR is recorded as antidromic action potentials at the ventral root. A drug which inhibits nerve-terminal function will reduce PTR and PTP.[10]

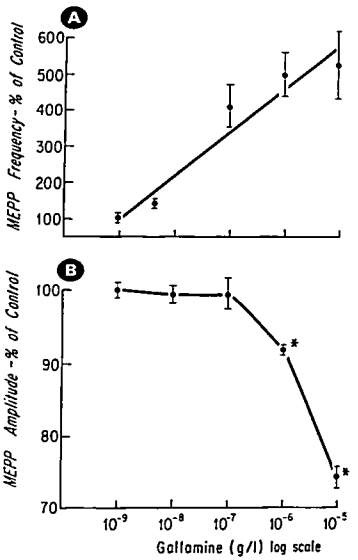

FIG. 4. Effects of gallamine, 10^{-9} to 10^{-5} g/l, on MEPP frequency and amplitude. *A*, frequency; *B*, amplitude. Values are means ±SE, $n = 10$, $P \leq 0.05$. Asterisks denote significant differences from control by Student's t test for paired data.

STATISTICS

For the determination of dose–response relationships, regression analysis by the method of analysis of variance was employed.[11] When the regression was significant and did not deviate from linearity, the best-fitting straight line was calculated and drawn. When there was a deviation from linearity, comparisons were made with Student's t test for paired data.[11] $P \leq 0.05$ was considered significant.

Results

Gallamine in concentrations of less than 10^{-9} g/l had no effect on the indirectly-stimulated

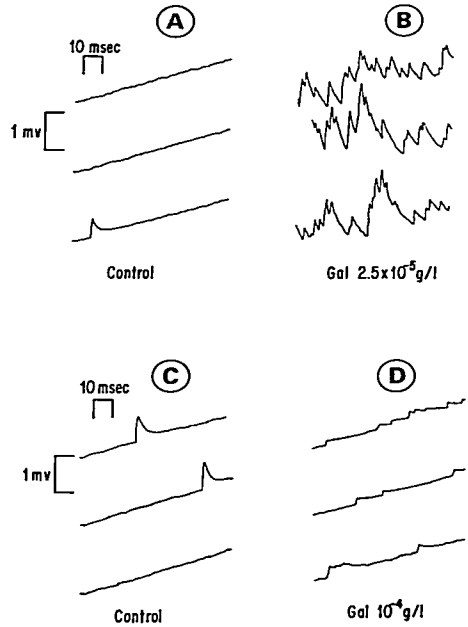

FIG. 5. Effects of gallamine, 2.5×10^{-6} and 10^{-4} g/l, on MEPP. *A*, control; *B*, 2 minutes after gallamine, 2.5×10^{-6} g/l; *C*, control; *D*, 2 minutes after gallamine 10^{-4} g/l. These tracings represent oscilloscope recordings photographed with a 35-mm camera and are typical records from the fibers in the muscles studied.

frog sartorius muscle. In a wide range of doses from 10^{-9} to 10^{-5} g/l, the agent produced 20–30 per cent increases in the force of contraction when it was added to the bath. Either the effect was transient or, as observed in most experiments, the contractions remained elevated until drug-free Ringer's solution was added to the bath. A typical record for one of the ten muscles tested at 10^{-6} g/l can be seen in figure 1. At 5×10^{-5} g/l, the drug produced a 7 per cent reduction in the twitch height. At 10^{-4} g/l, twitch height was reduced to 55 per cent of control, and at 5×10^{-4} g/l the force of contraction was reduced to 7 per cent of control (fig. 2). In every instance blockade was preceded by a period of stimulation of twitch height. Furthermore, two distinct patterns of blockade were noted. In the first, following the initial stimulation, there was a transient blockade followed by a period in which twitch height rose to much higher than control. It then began to decrease slowly, and blockade ensued. In the second pattern, following the initial stimulation, the normal blockade occurred. However, during blockade there were two or three periods during which twitch height was transiently increased slightly and then returned to the preceding or lower levels. Typical records of these two patterns can be seen in figure 3.

In concentrations from 10^{-9} to 10^{-5} g/l, gallamine produced a dose-related increase in MEPP frequency to 530 per cent of control. There was no effect on amplitude at 10^{-9} to 10^{-7} g/l. A dose of 10^{-6} g/l reduced MEPP amplitude to 93 per cent of control, and 10^{-5} g/l reduced it to 74 per cent of control (fig. 4). These effects were found within a few seconds of administration of the drug and remained as long as the agent stayed in contact with the

muscle. In some instances this was as long as two hours.

At a dose of 2.5×10^{-5} g/l, the increase in MEPP frequency was so great that it was impossible to make an accurate estimation of either frequency or amplitude. The amplitude was reduced, but since the MEPP's partially overlapped each other, it was impossible to determine adequately their exact shape, and hence their exact amplitude. Typical oscilloscope recordings can be seen in figure 5. Throughout the entire range of concentrations which blocked twitch height, namely 5×10^{-5} to 5×10^{-4} g/l, this marked increase in frequency was present, with a continual reduction in amplitude. At 10^{-4} g/l, the reduction in amplitude was such that the MEPP's began to become obscured by the inherent noise of the recording system. This can be seen in figure 5. However, there was no evidence that in this range MEPP frequency was reduced. Associated with the huge increase in frequency was a 10–15-mv reduction of resting membrane potential. It can be seen in figure 6 that the increase in frequency and depolarization occurred simultaneously. Washing the preparation with Ringer's solution for 10 minutes returned both to normal.

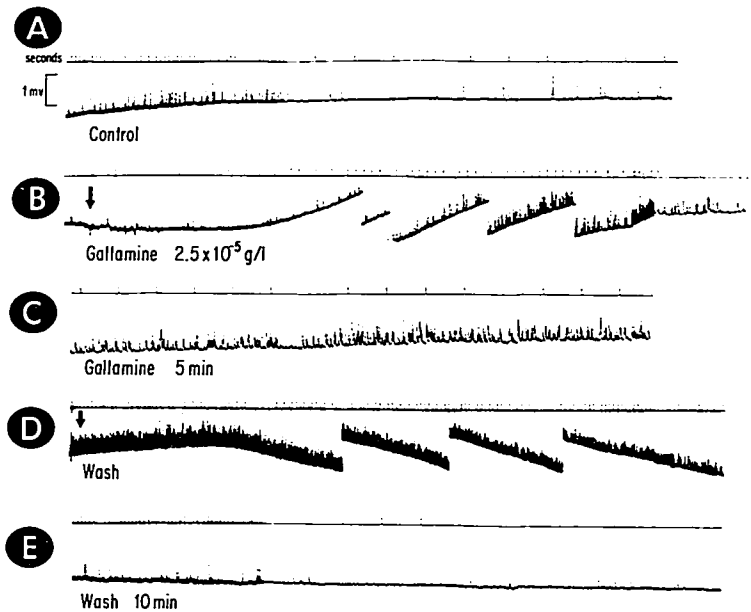

FIG. 6. Graphic recording of the effects of gallamine, 2.5×10^{-5} g/l, on MEPP and resting membrane potential. *A*, control. *B*, appplication of gallamine (arrow). Note depolarization and increased MEPP frequency. One-millivolt down-steps are used to keep the potential tracing on the chart. *C*, after application of gallamine for 5 minutes. *D*, washout in Ringer's solution (arrow). Note repolarization. *E*, after 10 minutes of washout. Note the stable potential and decrease in MEPP frequency to about the control level.

The time scale, top tracing of each pair, changes. These records were made with an Elema-Schonander Mingograph 81 recorder and are typical of the fibers in the muscles observed.

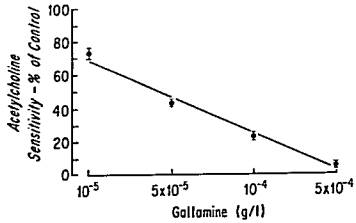

FIG. 7. Effects of gallamine, 10^{-5} to 5×10^{-4} g/l, on the sensitivity of denervated endplates to iontophoretically applied acetylcholine. Values are means ±SE, n = 10.

The effect of gallamine on the endplate alone was evaluated using denervated muscles. The appearance of small MEPP's indicates the proximity of the endplate region. Birks *et al.*[12] have shown that MEPP's are still present in denervated muscle. They have smaller amplitudes and their frequency is 1/100 of normal. When gallamine in a range of concentrations from 2.5×10^{-5} to 5×10^{-4} g/l was added to the bath, there was no alteration in the resting membrane potential and no increase in MEPP frequency. Thus, it would appear that the depolarization associated with the drug was neural in origin.

The sensitivity of the endplate region to focal iontophoretic application of acetylcholine was investigated in denervated muscle preparations exposed to gallamine between concentrations of 10^{-5} and 5×10^{-4} g/l. Innervated muscle was not used because the drug-induced reduction of the resting membrane potential to close to −70 mv would itself alter acetylcholine sensitivity as well as MEPP amplitude. At 10^{-5} g/l, sensitivity was reduced to 65 per cent of control. At 5×10^{-5} g/l, the concentration at which the twitch height was initially reduced, sensitivity was down to 40 per cent of control. Finally, at 5×10^{-4} g/l, the response to acetylcholine was almost completely inhibited (fig. 7).

When gallamine concentrations from 10^{-9} to 10^{-5} g/l were added to the bath around the sartorius muscle, twitch height increased. Associated with this was the appearance of antidromic action potentials (fig. 8). In the six muscles tested, these potentials varied considerably in number, amplitude, duration, and time interval from the orthodromic stimulus. The mean levels ranged from 100 to 300 μv in amplitude, with durations of 5–10 msec. Antidromic action potentials were seen so long as the drug-induced increases in twitch height were above control levels. Washing returned twitch height to normal and the antidromic potentials vanished.

When gallamine, in all concentrations which had reduced the height of the muscle twitch, was added to the bath, there was no alteration in either posttetanic potentiation (PTP) or posttetanic repetitive firing (PTR). Following tetatnus, these antidromic action potentials were still seen in the presence of gallamine, 10^{-4} g/l (fig. 9). Upon increasing the concentration to 10^{-3} g/l, PTP and PTR were finally abolished (fig. 9). The reduction in PTR was seen within two minutes of application of the drug, and a washout period of 10–15 minutes was needed for PTP and PTR to return.

Discussion

The data indicate that at concentrations well below those necessary for depression of twitch height gallamine has a stimulatory action on the nerve terminal. It increases MEPP frequency and produces repetitive firing of the nerve terminal in response to a single stimulus, concomitant with an increase in the force of contraction. Stimulation of the nerve terminal persists throughout the concentration range which produces myoneural blockade. Nerve-terminal function is still normal, as can be seen by the fact that PTP and PTR are still present. At a dose of 10^{-5} g/l there is no obvious decline in twitch height, yet MEPP amplitude and acetylcholine sensitivity are reduced by 30 per cent. In the dose range at which gallamine reduces twitch height, the sensitivity of the endplate region to acetylcholine is reduced to from 40 to 5 per cent of control. According to Fatt and Katz,[13] an endplate potential below 40 per cent of control is not sufficient to generate an action potential. Therefore, it must be concluded that blockade of the postsynaptic membrane is the mechanism whereby this drug produces failure of conduction. At very high doses, at which muscle twitch is completely inhibited, gallamine has

an inhibitory action on the motor nerve terminal. A concentration of 10^{-3} g/l inhibits both PTP and PTR.

The mechanism by which gallamine produces myoneural blockade differs from that of the other so-called "competitive blocking agents." *d*-Tubocurarine and pancuronium also stimulate the nerve terminal in low doses. However, in the range of concentrations at which they produce neuromuscular blockade these agents markedly inhibit nerve-terminal function, with only slight depression of the endplate. The action on the neuronal membrane is postulated to be the mechanism whereby curare and pancuronium inhibit neuromuscular conduction.[5, 14]

In recent years, it has become apparent that anticholinesterase agents, such as physostigmine and neostigmine, and depolarizing myoneural blockers, such as succinylcholine and decamethonium, have marked stimulatory actions on the nerve terminal.[15] In response to a single nerve action potential, the drugs cause the nerve terminal to fire repetitively and hence increase twitch height. This is referred to as "post-drug repetitive firing" (PDR). The appearance of antidromic action potentials recorded at the ventral root following the administration of small doses of gallamine is analogous to this PDR.

The pattern of neuromuscular blockade in which there are periods of stimulation during the decline of twitch height might be accounted for by the tremendous increase in MEPP frequency and repetitive firing of the terminal. At some point in time, enough acetylcholine might be released by huge stimulation of the terminal to overcome the postsynaptic blockade by gallamine, and more muscle fibers might fire to increase the overall muscle twitch height. In periods during which the terminal is not stimulated to the same degree, the postsynaptic blockade would prevail.

The reduction of the resting membrane potential by gallamine results from an effect on the nerve terminal that causes it to release large quantities of acetylcholine. The neurotransmitter produces the actual depolarization by acting on the endplate. This is verified by the fact that gallamine is devoid of a depolarizing effect on denervated muscle. It has been shown that the so-called "depolarizing" neuromuscular blocking agents, succinylcholine and decamethonium, depolarize the endplate directly.[16] In addition, they too cause massive release of acetylcholine from the nerve terminal.

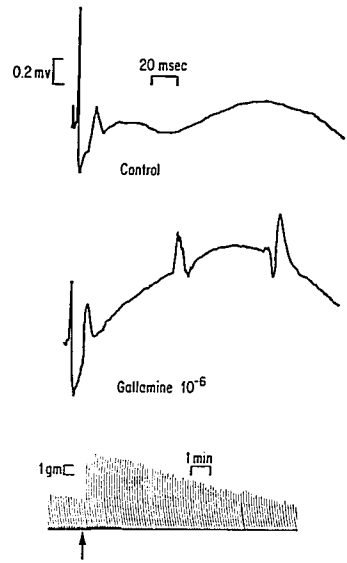

FIG. 8. Effects of gallamine, 10^{-4} g/l, on twitch height and nerve-terminal activity recorded from the ventral root. *Top trace*, nerve-terminal activity recorded from the ventral root following stimulation of the nerve in drug-free Ringer's solution. *Middle trace*, nerve-terminal activity following stimulation of the nerve in the presence of gallamine, 10^{-4} g/l. *Bottom trace*, record of muscle twitch. The arrow indicates when gallamine was added to the bath. These recordings are typical of those obtained from six frogs.

In the original investigation of gallamine, Riker and Wescoe [2] found that in low doses it increased the force of contraction of the indirectly-stimulated gastrocnemius muscle of the cat. Jones and Laity [17] also described the increased twitch height produced by low doses, and ascribed this to an action on the motor nerve terminal. Patton and Shand [18] reported that the drug stimulated the nerve

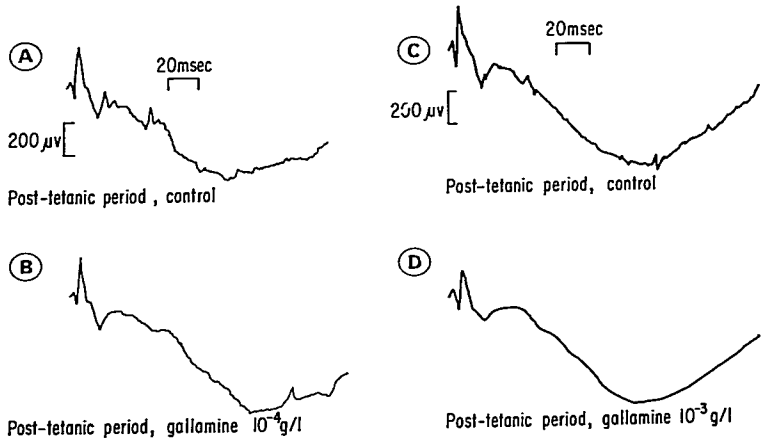

FIG. 9. Effects of gallamine, 10^{-4} and 10^{-3} g/l, on posttetanic repetitive activity in the nerve terminal. *A*, nerve-terminal activity recorded at the ventral root following stimulation of the nerve after a conditioning period of a 10-second tetanus. *B*, nerve-terminal activity following stimulation of the nerve after a conditioning period of a 10-second tetanus in the presence of gallamine, 10^{-4} g/l. *C*, nerve-terminal activity recorded at the ventral root following stimulation of the nerve after a conditioning period of a 10-second tetanus. *D*, nerve-terminal activity following stimulation of the nerve after a conditioning period of a 10-second tetanus in the presence of gallamine, 10^{-3} g/l. These recordings are typical of those obtained from six frogs. The frequency of the conditioning tetanus was 10 Hz. *A* and *B* are from one preparation and *C* and *D* are from another.

terminal while it reduced MEPP amplitude in the frog sartorius muscle. Bulbring and Depierre[19] showed that gallamine blocked the responsiveness of denervated cat muscle to acetylcholine.

This investigation confirms previous reports that gallamine has a dual action on the neuromuscular junction. It produces massive stimulation of the terminal at the same time that it blocks the endplate. However, at very high doses, it does inhibit nerve-terminal function. This type of dual action has been seen with other drugs at other locations. Tetraethylammonium blocks the action of acetylcholine on the postsynaptic membrane of isolated perfused ganglia and adrenal medulla while it enhances presynaptic release.[20] At the neuromuscular junction, triethylcholine also has this dual action.[21]

The combination of *d*-tubocurarine and gallamine has been shown to lead to potentiation of neuromuscular blockade rather than just simple addition. Wong and Jones[22] reported a potentiating action of the two drugs in the rabbit and in the mouse. Ghoneim *et al.*[23] have recently shown that this effect also occurs in man and in the dog, and is caused by an interaction at the neuromuscular junction specifically. This potentiation might be accounted for by the fact that whereas gallamine primarily affects the endplate, *d*-tubocurarine has depressant effects on both pre- and postjunctional membranes.

It is concluded that gallamine has three distinct effects on neuromuscular conduction: 1) at low doses there is nerve-terminal stimulation; 2) at higher doses the sensitivity of the endplate to acetylcholine is reduced concomitant with reduction in twitch height; 3) at the highest doses studied, nerve-terminal function is inhibited.

The authors thank Mr. L. R. Davies for expert technical assistance and Lederle Laboratories for providing the gallamine used in this study.

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