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The Actions of Neuromuscular Relaxants at Hyperbaric Pressures

Calypso Gountis-Bonikos, M.D., Joan J. Kendig, Ph.D.,† Ellis N. Cohen, M.D.‡*

Previous studies have shown that high pressures increase the twitch tension of directly or indirectly stimulated mammalian muscle, while depressing transmission at the neuromuscular junction. The present paper explores the interaction between high pressures and muscle relaxants on the indirectly stimulated rat phrenic nerve–diaphragm preparation. Two muscle relaxants, *d*-tubocurarine and succinylcholine, were studied.

Measurements of the electromyogram and twitch tension amplitudes were made before and after application of the muscle relaxants and compared with results of measurements of those two variables under hyperbaric conditions. High pressures tended to enhance neuromuscular blockade by *d*-tubocurarine more than that by succinylcholine, as indicated by electromyographic suppression, and high pressures tended to antagonize the succinylcholine effect on twitch tension more than that of *d*-tubocurarine. The findings are in agreement with previous observations that high pressures increase muscular twitch tension and depress excitatory synapses. They further demonstrate a complex interaction between hyperbaric pressures and neuromuscular blocking drugs. (Key words: Neuromuscular relaxants, *d*-tubocurarine; Neuromuscular relaxants, succinylcholine; Hyperbaria, neuromuscular transmission.)

ANESTHESIOLOGISTS have been interested in the study of physiologic and pharmacologic effects of high pressures since the demonstration of pressure reversal of anesthesia.[1–3] Pressure reversal of anesthesia is one example of the effects of high pressures on excitable tissue and of the interaction between drugs and hyperbaric pressures. A further example was provided by studies of skeletal muscle wherein pressure-associated changes in muscle contraction were among the earliest observations made in hyperbaric physiology.[4] Spontaneous contraction and increase in evoked twitch tension with pressure have been observed by several investigators.[5–8] At least one report has described the interactions between various drugs and pressure in cat skeletal muscle *in vivo*.[9] Inhibition of crustacean neuromuscular transmission at hyperbaric pressures has also been reported,[10] and pressure–anesthetic interactions have been examined in an isolated mammalian nerve–muscle preparation.[11]

* Clinical Assistant Professor of Anesthesia.

† Assistant Professor of Biology in Anesthesia.

‡ Professor of Anesthesia.

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Address reprint requests to Dr. Gountis-Bonikos.

The purpose of the present study was to investigate the mode of interaction between high pressures and muscle relaxants on mammalian neuromuscular function. Two representative muscle relaxants, *d*-tubocurarine (*d*Tc) and succinylcholine, were chosen. The two drugs block neuromuscular transmission by different modes of action, and it was thought they might manifest different interactions with pressure.

Methods

The hemidiaphragm and phrenic nerve were removed from male Sprague-Dawley rats, weights 250–400 g, that had been anesthetized with sodium pentobarbital, 60 mg/kg, intraperitoneally. The diaphragm was placed in Krebs–bicarbonate solution (NaCl 118mM; $NaHCO_3$, 25 mM; KCl 4.7 mM; KH_2PO_4 1.2 mM; $MgSO_4$ 1.2 mM; $CaCl_2$ 2.5 mM; glucose 11 mM), equilibrated with a mixture of 95 per cent O_2 and 5 per cent CO_2, and maintained at 34 ± 0.5 C throughout the study. Bath volume was 100 ml. After having been trimmed to a segment approximately 1 cm wide, centered on the phrenic nerve insertion and including ribs and central tendon, the diaphragm was fastened to a Plexiglass holder at the costal margin and to a strain gauge by a hook through the central tendon. Resting tension was adjusted to 2.5 g. Stimulating electrodes were placed on the phrenic nerve; the muscle compound action potential (electromyogram, EMG) was recorded by a wire sutured to the tissue, insulated except at one point where it passed through the muscle. The reference electrode was a silver–silver chloride wire immersed in the Krebs' solution. Stimuli consisted of pulses 0.2 msec in duration delivered by an isolated stimulator at a constant frequency of 0.5 or 0.2 Hz throughout the experiment. Stimulus intensity was adjusted to a value two to four times that which produced a maximum EMG response. Muscle twitch tension was recorded on a strip chart recorder. EMG responses were amplified, displayed on an oscilloscope screen, and photographed.

Recording was begun after the preparation had been in place for an hour, at which time both tension and EMG were stable. Stock solutions of the muscle relaxants were diluted in oxygenated Krebs' solution and added to each diaphragm–phrenic nerve preparation in concentrations so as to depress the EMG and twitch tension amplitude to approximately 50 per cent of control levels. The required concentrations for *d*Tc ranged from 7.1

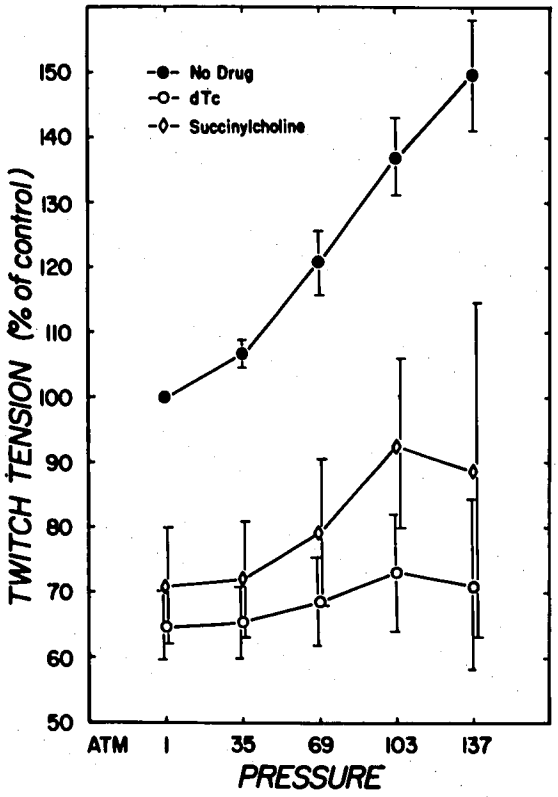


FIG. 1. Effects of hyperbaric pressures on the twitch response of the indirectly stimulated phrenic nerve–diaphragm preparation. Each point represents results from six preparations. Vertical bars are standard errors of mean (SEM). Control was the value at 1 ATA with no drug present. The enhancement of twitch response when no drug was present was significant at 137 ATA ($P < 0.01$). In the presence of succinylcholine, the twitch tension was significantly enhanced at 103 ATA and 137 ATA ($P < 0.05$). In the presence of *d*Tc, pressure tended to enhance twitch tension, but the increase was not significant.

$\times 10^{-7}$ to 1.4×10^{-6} M; those for succinylcholine ranged from 2.2×10^{-6} to 4.4×10^{-6} M

Equilibration of muscle relaxant and tissue was considered complete when records taken 5 minutes apart showed no further change in EMG or twitch tension amplitude. Pressure was applied by placing the bath containing the preparation in a stainless steel pressure vessel of approximately 1 liter capacity with provision for electrical connections. The vessel was flushed with the 95 per cent O_2/5 per cent CO_2 gas mixture, and helium admitted from a cylinder pressurized to 3,500 psi. The *p*H of the bathing medium was measured in separate experiments by a modified glass *p*H electrode and standard reference electrode; on compression to 200 atmospheres absolute (ATA), the *p*H of the Krebs' solution decreased 0.2 units, returning to its previous value on decompression. This change is consistent with pressure-related changes in the bicarbonate and phosphate buffer dissociation constants.[12] Pressure was applied in increments of 500 psi (35 ATA), each increment being achieved in less than a minute. Temperature was regulated by a water bath in which the pressure vessel was immersed. Muscle tension was recorded continuously; EMG responses were recorded 30 seconds after pressure had stabilized. Decompression from 137 ATA to 1 ATA was accomplished rapidly and continuously, and was complete within 2 minutes.

Both twitch tension and EMG responses were measured as amplitudes from baseline, and displayed as percentages of control amplitude (amplitude at 1 ATA pressure with no muscle relaxant present). In all experiments, t tests for paired data were used to assess the significance of differences among control amplitude, amplitude at 1 ATA with muscle relaxant, and amplitude under pressure.

Results

Effects of Pressure on the Untreated Muscle

Pressure enhanced the twitch response of the indirectly stimulated rat diaphragm by approximately

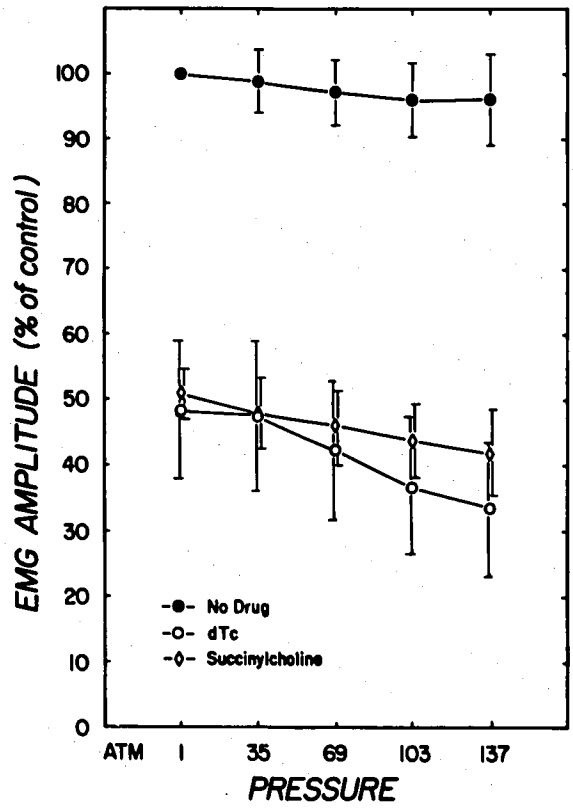
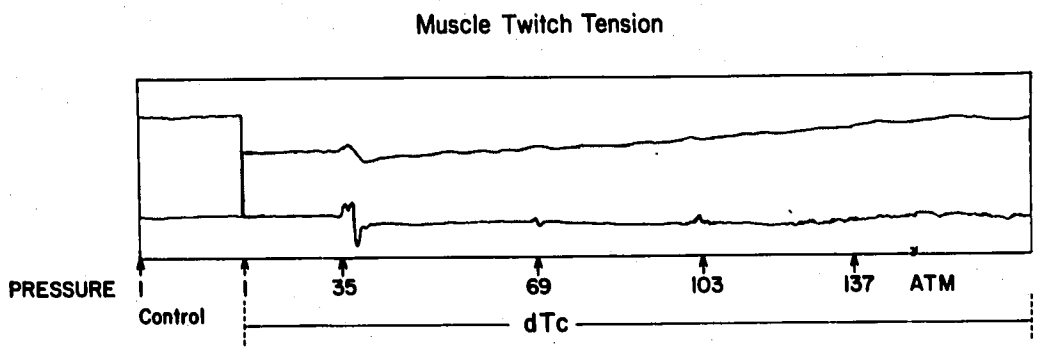


FIG. 2. Effects of hyperbaric pressures on the EMG of the indirectly stimulated phrenic nerve–diaphragm preparation. Each point represents results from six preparations. Vertical bars are standard errors of the mean (SEM). Control was the value at 1 ATA with no drug present. There was no change of the EMG amplitude with no drug present. In the presence of succinylcholine, high pressures tended to depress EMG amplitude, but the depression was not significant. In the presence of *d*Tc, high pressures depressed the EMG; this was significant at 103 ATA and 137 ATA ($P < 0.05$).



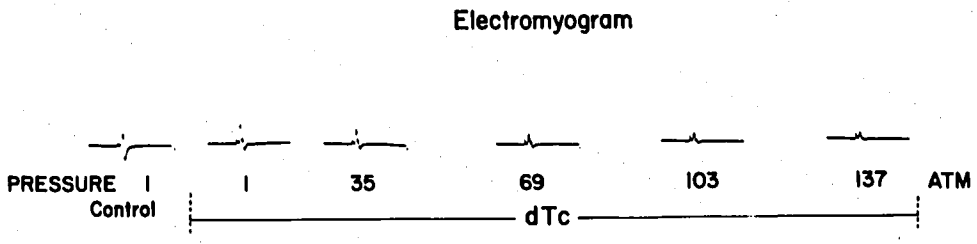
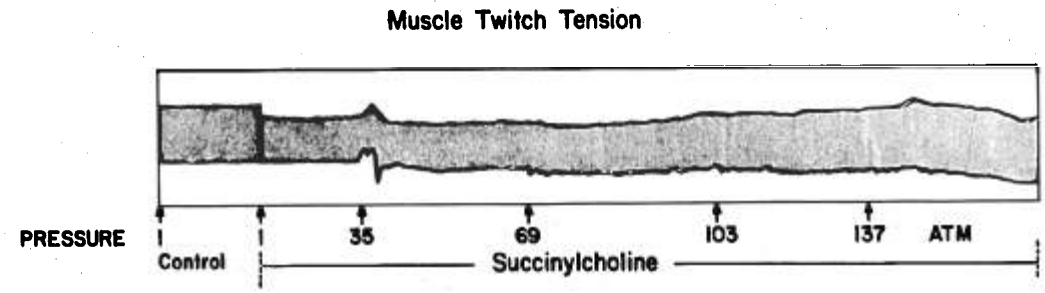


FIG. 3. Effects of hyperbaric pressures on the indirectly stimulated phrenic nerve – diaphragm preparation when *d*Tc was added. *Above*, twitch tension; full scale is equal to 10 g tension. *Below*, EMG. The effects of pressure were reversible on decompression. *d*Tc concentration, 7.1×10^{-7} M.



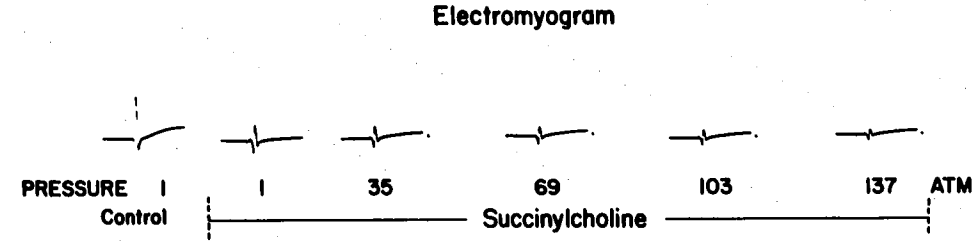


FIG. 4. Effects of hyperbaric pressures on the indirectly stimulated phrenic nerve–diaphragm preparation when succinylcholine was added. *Above*, twitch tension; full scale is equal to 10 g tension. *Below*, EMG. The effects of pressure were reversible on decompression. Succinylcholine concentration, 2.2×10^{-6} M.

50 per cent at 137 ATA ($P < 0.01$) (fig. 1). This result has been reported previously. Also as reported, the increase in twitch tension was not accompanied by any change in amplitude of the EMG (fig. 2). Since the twitch tension increase is also observed in directly stimulated muscles, this effect of pressure is due to a direct action on the muscle itself, rather than on the nerve or the neuromuscular junction.

PRESSURE–MUSCLE RELAXANT INTERACTIONS

The concentrations of *d*Tc and succinylcholine were adjusted to depress EMG amplitude by approximately 50 per cent at 1 atmosphere. The corresponding twitch heights averaged 65 per cent of control for *d*Tc and 71 per cent of control for succinylcholine (fig. 1–3). Depressions of both variables were significant ($P < 0.01$).

Compression to 137 ATA tended to depress EMG amplitude but enhance twitch height in preparations treated with either drug (fig. 3 and 4). The pressure-related depression of EMG amplitude was significant in preparations treated with *d*Tc ($P < 0.05$), but not in those treated with succinylcholine (fig. 2). Conversely, the enhancement of twitch tension by pressure was significant in preparations treated with succinylcholine ($P < 0.05$), but not those treated with *d*Tc (fig. 1).

Upon decompression to 1 ATA, EMG and twitch tension returned rapidly towards control values in all experiments (not shown). Decompression was associated with oxygen loss from the bathing medium. When the medium was re-equilibrated

with 95 per cent O_2/5 per cent CO_2, return to control levels was complete.

Discussion

In the untreated preparation, pressure enhanced twitch tension but did not change the EMG response to stimulation of the phrenic nerve. The effects of high pressure on the neuromuscular preparation were modified by the use of muscle relaxants. In preparations treated with *d*Tc, pressure added to the EMG depression produced by the drug (figs. 2 and 3). Although twitch tension still tended to increase with pressure, the increase was not significant in curarized preparations (figs. 1 and 3). In preparations treated with succinylcholine, on the other hand, high pressures depressed the EMG slightly but not significantly (fig. 2) and did enhance twitch tension, though to a lesser extent than the twitch tension enhancement observed in untreated preparations (figs. 1 and 4). The interactions between pressure and the two relaxants are thus qualitatively similar, with pressure tending to depress the EMG but enhance twitch tension. The first effect is more marked with *d*Tc and the second with succinylcholine.

These results are consistent with those of a previous study[11] in which pressure depressed the EMG response of the indirectly stimulated diaphragm exposed to a low calcium concentration, but had no effect on the EMG recorded when the diaphragm was exposed to a physiologic calcium concentration. The results of both studies suggest that hyperbaric pressures depress transmission at the neuromuscular junction. In the rat diaphragm, pressure to 137 ATA is sufficient to block transmission only when the safety factor for transmission has been decreased by partial "curarization" or treatment with a low calcium concentration.

Hyperbaric depression of excitatory synaptic transmission has now been observed at four sites, the mammalian skeletal neuromuscular junction,[11] the crustacean neuromuscular junction,[10] the synapse between mammalian pre- and postganglionic sympathetic neurons,[13] and the squid giant synapse.[14] The basis for the phenomenon has not been established, although there is indirect evidence that pressure may inhibit transmitter release.[10] The enhancement of twitch tension by pressure occurs at a muscle site other than the neuromuscular junction since it can be observed in the directly stimulated preparation.[11] The two effects are thus distinct and unrelated.

That *d*Tc and succinylcholine should interact somewhat differently with pressure is not surprising, since the two drugs show distinct pharmacologic differences. *d*Tc belongs to the class of nondepolarizing muscle relaxants that act as competitive antagonists to acetylcholine. Succinylcholine, on the other hand, acts as a partial agonist to acetylcholine, producing a neuromuscular blockade characterized as either "depolarizing" or "desensitizing" depending on the preparation and the duration of exposure to the agent. We do not know which type of succinylcholine blockade was present in our preparation.

The results of the present experiments, as seen in the EMG records, suggest that high pressure enhances blackade by *d*Tc more than blockade by succinylcholine. A plausible explanation of why this may be so rests on two pieces of indirect evidence. First, as described above, experiments on another preparation[10] suggest that the effect of pressure is to diminish the amount of transmitter released at the neuromuscular junction. Second, it is known that *d*Tc-induced blockade, but not succinylcholine-induced blockade, is antagonized by increasing the amount of acetylcholine, *e.g.*, by inhibiting cholinesterase. The opposite interaction, an enhancement of nondepolarizing blockade by a decrease in the amount of acetylcholine, is widely assumed. If indeed pressure inhibits transmitter release, the enhancement of *d*Tc-induced blockade under hyperbaric conditions may be explained on these grounds.

Changes in twitch tension under hyperbaric conditions are in the direction opposite to EMG changes, which indicates that high pressure exerts two separate effects on neuromuscular function. One is the already-described depression at the neuromuscular junction, the other an enhancement of some process subsequent to muscle excitation, either excitation–contraction coupling or the contractile process itself. The result of these opposing effects of pressure is an apparent antagonism between neuromuscular blocking drugs and pressure when twitch height is used as the index of blockade. This antagonism is opposed by the additive effects of pressure and neuromuscular blocking drugs at the neuromuscular junction. The relative importances of these separate opposing effects are predicated to depend on the blocking dose used; obviously no twitch will be observed when block at the junction is complete. Although it will obviously be necessary to confirm this finding in the intact animal, the results suggest caution in the use of neuromuscular blocking drugs at high pressures, *e.g.*, in deep-sea-diving accidents when treatment must be carried out at high pressures. These results also point up the complexity of pressure–drug interactions, and the necessity for further research.

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Myocardial Infarction

NITROPRUSSIDE AND NITROGLYCERIN Both nitroglycerin (TNG) and nitroprusside (NP) reduce afterload and therefore might be expected to be beneficial in patients with impaired coronary blood flow. The authors evaluated ten patients who had acute transmural anterior infarction. Pulmonary arterial pressure, pulmonary capillary wedge pressure, and systemic arterial pressure were monitored. Precordial electrocardiograms (V_1–V_6) were examined and the lead with maximal ST-segment stability was assessed. NP was administered intravenously (average rate 95 μg/min). In five patients, NP was discontinued and, 15 minutes later, when the hemodynamic effect had disappeared, TNG was administered (0.3 or 0.6 mg, sublingually). Administration of NP reduced arterial pressure from 105 ± 8 (SE) to 82 ± 5 torr and wedge pressure from 19 ± 1 to 12 ± 1 torr. Cardiac rate increased from 83 ± 5 to 93 ± 5/min. Administration of TNG resulted in a decrease in arterial pressure from 112 ± 9 to 98 ± 10 torr and wedge pressure from 21 ± 4 to 12 ± 4 torr. Although the two drugs had similar effects on arterial and wedge pressures, changes in ST-segment elevations were strikingly different. Following NP, average ST-segment elevation rose by 2.4 ± 0.3 mm. Nitroglycerin reduced ST-segment elevation by 1.4 ± 0.4 mm. Further observations were made in 14 anesthetized (thiamylal, 25 mg/kg, iv) and ventilated mongrel dogs after occlusion of the left anterior descending coronary artery. In seven dogs, NP was infused intravenously for 30 minutes beginning 15 minutes after occlusion. Following a 5-minute rest, TNG was administered as a bolus, followed by constant infusion. In addition to monitoring the epicardial electrogram, regional myocardial blood flow (RMBF) was determined by microsphere technique. Again, NP increased and TNG reduced ST-segment elevation. NP reduced RMBF by 23 per cent in the ischemic zones; administration of TNG resulted in a flow higher than both NP and control values. The ratio of endo/epicardial flows in the ischemic zone did not change after administration of NP but increased after TNG was administered. The authors conclude that TNG reduces electrocardiographic ischemic injury "by increasing perfusion of the ischemic areas and redistributing it favorably, while NP increased electrogradiographic ischemic injury, at least in part, by reducing perfusion." *(Chiariello M, and others: Comparison between the effects of nitroprusside and nitroglycerin on ischemic injury during acute myocardial infarction. Circulation 54:766–773, 1976.)*