The Effects of Hexafluorenium and Edrophonium on the Neuromuscular Blocking Actions of Succinyicholine, Decamethonium, Imbretil and d-Tubocurarine

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Hexafluorenium had no effect on the twitch response in anesthetized man. The phase 1 depolarizing or phase 2 nondepolarizing block produced by succinylcholine was increased by the injection of hexafluorenium. Edrophonium under similar circumstances increased the phase 1 depolarizing block, but antagonized the phase 2 nondepolarizing block. The phase 1 depolarizing block produced by decamethonium or Imbretil was antagonized by hexafluorenium, while the phase 2 nondepolarizing block produced by decamethonium or Imbretil was potentiated by hexafluorenium. Edrophonium potentiated the phase 1 depolarizing block of decamethonium and Imbretil and antagonized the phase 2 nondepolarizing block of decamethonium and Imbretil. The d-tubocurarine block was potentiated by hexafluorenium and antagonized by edrophonium. The results with edrophonium were attributed to inhibition of true cholinesterase. The effects of hexafluorenium were attributed to its junctional membrane, nondepolarizing and pseudocholinesterase inhibiting actions.

Preliminary studies with hexafluorenium (Mylaxen) suggested that this agent (1) had a weak curare-like effect, (2) potentiated the action of succinylcholine (SCh) by means of an anti-pseudocholinesterase action and (3) had little effect on blood pressure, heart rate, cardiac rhythm or bronchial tone.1-5

Subsequent studies were not in agreement with all these findings. Nastuk and Karis6 agreed that hexafluorenium (HFL) and d-tubocurarine (dTC) appear similar in that both block neuromuscular transmission without any evidence of facilitation or direct muscle stimulation. However, they pointed out that these 2 drugs differ in other effects and the conclusion that they have parallel actions is not necessarily warranted. Nastuk and Karis6 also stated that although inhibition of plasma cholinesterase (pseudocholinesterase) might partly account for the in vitro potention of SCh, it cannot account for the in vitro potention of SCh by HFL. They therefore suggested an alternate mode of action to explain the results of their in vitro experiments and which may also explain in vivo potentiation (presented in discussion).

Selvin and Howland7 reported that HFL and SCh produced bronchospasm in 6 patients with one fatality. Mostert8,9 found that HFL produced bronchospasm, tachycardia, a fall in blood pressure, cardiac arrhythmias and antagonism of the neuromuscular block produced by Imbretil.

Because of these conflicting data, the following study was undertaken to determine the effects of HFL in man and to compare them with edrophonium (Tensilon), an inhibitor of red cell cholinesterase (true cholinesterase).

Methods

Sixty patients were studied during anesthesia and operation. Most of the patients were medicated with atropine or scopolamine 0.4-0.8 mg., pentobarbital 100 mg. and/or meperidine 50-100 mg. Anesthesia was usually induced with 150-500 mg. of 2.5 per cent thiomylal and maintained with nitrous oxide, supplemented in some cases by thiomylal or meperidine. Endotracheal intubation was carried out, where necessary, following topical anesthesia with 3 ml. of 4 per cent lidocaine or after the injection of 20-60 mg. of SCh. The results in the patients intubated following topical anesthesia did not differ from those intubated with the aid of succinylcholine. Ventilation was measured with a Wright ventilometer or a Collin Respirometer. In the former case, a circle system with a 3-8 liter flow and a carbon dioxide absorber was used.

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while in the latter a nonbreathing system utilizing a Sierra nonbreathing valve (28–560) was used. Respiration was spontaneous, assisted, or controlled, as required. Lead 2 of the electrocardiogram was monitored. After the induction dose of thiopental, but before any muscle relaxant had been given, the patient’s hand was carefully fixed in place on a specially constructed molded plaster armboard. A supramaximal stimulus (100–150 volts) from a Grass stimulator (model SC4) and a stimulus isolation unit was applied to the ulnar nerve at the wrist by means of subcutaneously placed 25-gauge needle electrodes. In some patients the ulnar nerve was stimulated at the elbow. Twitch responses were elicited with square pulse stimuli of 0.3 msec. duration delivered at a rate of 0.3 cycles/second (18/min.). Tetanus was obtained by stimulating at 30 cycles/sec. for 2–3 seconds. The resulting adduction of the thumb was measured by a force displacement transducer (Grass FT-03) and continuously recorded on an Invengineering polygraph utilizing Offner components. The sensitivity of the system was adjusted so that the control twitch height was a minimum of 30 mm. and a maximum of 50 mm. Each patient served as his own control. The nature of the block, i.e., phase 1 depolarizing or phase 2 nondepolarizing, was determined by the response to tetanus at a twitch height less than 20 per cent of the initial value. The block was considered depolarizing in nature if tetanus was well maintained and there was no post-tetanic facilitation. The block was considered to be nondepolarizing if the response to tetanus was not well maintained (Wedensky inhibition) and post-tetanic facilitation was significant.

Post-tetanic facilitation was defined as significant if the first twitch following tetanus was of a magnitude 2 or more times greater than the twitch immediately preceding the tetanus.10 Solutions of 2 per cent Sch, 0.3 per cent dTC, 0.1 per cent decamethonium, 0.1 per cent Imbretil, 2 per cent HFL and 1 per cent edrophonium were used. HFL 0.5 mg./kg. and edrophonium 0.15 mg./kg. were used except where otherwise indicated.

We also carried out a clinical study in 100 patients of the effects of HFL followed by Sch (HFL-Sch) under a variety of anesthetic techniques and operations. In these patients 0.5 mg./kg. of HFL was given, before or after induction of anesthesia. Three to five minutes later 0.2–0.3 mg./kg. of Sch was injected. Subsequent injections of 0.15–0.2 mg./kg. of Sch were made as necessary. When the effect of HFL began to wear off, another dose of 0.2–0.3 mg./kg. of HFL was given and followed by 0.1–0.2 mg./kg. of Sch, as required by the clinical situation. In 25 of these 100 patients the neuromuscular blocking effects of HFL and Sch were assessed with the aid of the nerve stimulator.

**Results (Table 1)**

**Hexafluorenium.** The injection of 0.5–1 mg./kg. of HFL without prior injection of a muscle relaxant had no effect on twitch height regardless of the anesthetic agent used.

**Succinylcholine.** The injection of 0.3 mg./kg. of Sch markedly decreased or abolished the twitch response. During recovery, tetanus was well maintained and post-tetanic facilitation was absent (i.e., phase 1 depolarizing block). Under these circumstances the injection of either HFL (fig. 1) or edrophonium

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increased the intensity of the block. In another group of patients 3–7 mg./kg. of SCh was given over 30–60 minutes by intermittent injection or continuous infusion. Wedensky inhibition (poorly sustained tetanus) and posttetanic facilitation were present (i.e., phase 2 nondepolarizing block). Under these circumstances the injection of HFL also increased the intensity of the block. The injection of edrophonium under similar circumstances antagonized the block. In summary, HFL potentiated both the phase 1 depolarizing and phase 2 nondepolarizing block, while edrophonium potentiated a phase 1 depolarizing and antagonized a phase 2 nondepolarizing block.

If HFL (0.5–1 mg./kg.) preceded the SCh (0.4–1 mg./kg.) a phase 2 nondepolarizing block was observed. This block was potentiated by HFL and antagonized by edrophonium.

Decamethonium. The injection of 25–30 µg./kg. of decamethonium markedly decreased twitch height. The interposition of 2–3 seconds of tetanus revealed a phase 1 depolarizing block. The injection of HFL antagonized the block (fig. 2) while edrophonium potentiated the block (fig. 3). Another group of patients was given 80–90 µg./kg. of decamethonium over 40–70 minutes until a phase 2 nondepolarizing block was established. The injection of HFL now potentiated the block (fig. 4) while edrophonium antagonized the block (fig. 5).

Hexamethylene Carbaminoethylcholine (Imbretil). The injection of 15–30 µg./kg. of Imbretil decreased twitch height. The response to tetanus demonstrated a phase 1 depolarizing block. The injection of HFL antagonized the block while edrophonium potentiated the block. Another group of patients was given 100 µg./kg.
kg. of Imbretil over 60 minutes until a phase 2 nondepolarizing block was produced. The injection of HFL now potentiated the block while edrophonium antagonized the block.

_d-Tubocurarine_. After establishing a neuromuscular block with dTC, HFL potentiated the block (fig. 6) while edrophonium antagonized the block.

**Other Observations.** The changes in respiration were usually similar to those in twitch height. In some of the patients ventilation could not be studied because of apnea. In other patients, despite a marked depression of twitch response, minimal changes in ventilation occurred. In those patients in whom ventilation was studied, tidal volume increased when the block was antagonized and decreased when the block was potentiated. The respiratory studies were usually carried out during a relatively steady state of anesthesia and in the absence of surgical stimulation.

HFL increased the pulse rate an average of 25 per cent, but sometimes as much as 100 per cent. The blood pressure frequently decreased, but occasionally increased. The magnitude of change was usually less than 15 per cent. Ventricular arrhythmias were not seen in the patients anesthetized with nitrous oxide and were rarely seen in patients given cyclopropane, halothane or trichlorethylene. An increase in oropharyngeal secretions was observed. Patients who received the drug while awake frequently complained of nausea and occasionally vomited. A minor to moderate degree of bronchospasm was occasionally seen during the use of the HFL-SCh sequence. This produced some difficulty in assessing the degree of relaxation. Although the reservoir breathing bag was "tight," the patient was well relaxed as judged by the surgeon and the twitch response recording.

**Discussion**

It has been reported by Cordaro and Arrowood that HFL is capable of producing relaxation adequate to permit abdominal sur-
Surgery in patients during light general anesthesia. Foldes et al.\textsuperscript{1,2} reported that 0.3–0.5 mg./kg. had a weak curare-like action in man. These studies were carried out at a time when it was customary to assess the action of a neuromuscular blocking agent by the effect on respiration, the “feel of the reservoir breathing bag” or the clinical impression of the surgeon or anesthesiologist. However, recent studies have shown that factors other than neuromuscular block affect these parameters and that assessing a neuromuscular block by respiratory effects and clinical impressions may be inaccurate.\textsuperscript{10} The technique of measuring the response of a muscle to indirect stimulation (nerve stimulation) has made possible a more quantitative analysis of the effects of the neuromuscular blocking agents. Using such a technique, 0.5–1 mg./kg. of HFL produced no neuromuscular block in the present experiments. Since it is known that the magnitude of neuromuscular blocking action of HFL is quite variable from species to species, (equal to dTC in the dog, greater than dTC in the rabbit, and less than dTC in the mouse\textsuperscript{11}) it is likely that a neuromuscular blocking action of HFL might have been demonstrated in man with the use of larger doses. This study was not attempted because of undesirable effects produced by HFL.

In the studies in which a neuromuscular blocking action of HFL was observed, the block was described as curare-like in nature because of the lack of preliminary fasciculation and the antagonism of the HFL block by neostigmine.\textsuperscript{11} Nastuk and Karis\textsuperscript{6} using an \textit{in vitro} indirectly stimulated frog sartorius prepa-ration also demonstrated a block of neuromuscular transmission by HFL which was similar to that seen with dTC. However, they pointed out that the dTC and HFL blocks differed in several respects. The HFL block could not be antagonized by edrophonium. Although both dTC and HFL prevented depolarization of the postjunctional membrane by acetylcholine, the mechanisms by which this occurred were different. They suggested that HFL blocks the mechanism by which the membrane becomes depolarized when receptor activation has occurred, unlike dTC which competes with acetylcholine for occupation of the receptor site. Since the dTC and HFL blocks are similar in some aspects and differ in others, the HFL block will be referred to as nondepolarizing rather than curare-like.

In the present study the injection of HFL after SCh potentiated the block regardless of the nature of the SCh block (phase 1 depolarizing or phase 2 nondepolarizing) or the order of injection of HFL and SCh. HFL is believed to potentiate the activity of SCh by inhibiting the action of plasma cholinesterase (pseudocholinesterase) thereby inhibiting the enzymatic breakdown of SCh.\textsuperscript{1,2,5} Although the anti-pseudocholinesterase action of HFL can explain the \textit{in vivo} potentiation of SCh, this explanation does not hold for the \textit{in vitro} potentiation of SCh found by Nastuk and Karis.\textsuperscript{6} They suggested that a drug capable of blocking membrane depolarization itself, but which permits receptor activation-inactivation to go on as usual during the sustained application of a depolarizing agent would enhance the blocking action of the depolarizer.
The relative importance of this junctional membrane effect of HFL and the anti-pseudocholinesterase effect on the total action of HFL in man is not yet known. Whether the junctional membrane effect and the nondepolarizing block of HFL are the same or related effects is also not yet known.

Although both HFL and edrophonium are anticholinesterases, the mechanisms of potentiation of a SCh phase 1 depolarizing block by these agents differ. HFL potentiates by inhibiting pseudocholinesterase (therefore decreasing the enzymatic breakdown of SCh) and/or by its junctional membrane effects. Edrophonium, however, inhibits true cholinesterase and therefore decreases the enzymatic breakdown of acetylcholine. The depolarizing action of acetylcholine added to that of a SCh phase 1 depolarizing block results in the increased activity of SCh. In view of the different modes of action of HFL and edrophonium, the different effects of these two agents on a phase 2 nondepolarizing succinylcholine block are to be expected. Regardless of the nature of the block, HFL will potentiate the SCh block by inhibiting plasma cholinesterase and/or by its effect at the junctional membrane. However, in a phase 2 nondepolarizing block produced by SCh, edrophonium will antagonize the block.\(^10\)

HFL and edrophonium also produced different effects on a dTC block, phase 1 and phase 2 decamethonium block, and phase 1 and phase 2 Imbretil block. Edrophonium potentiated the phase 1 depolarizing block of decamethonium and Imbretil and antagonized the nondepolarizing dTC and phase 2 nondepolarizing block of decamethonium and Imbretil. These results are similar to those listed above for the depolarizing and nondepolarizing phase of activity of SCh. In essence edrophonium potentiated a depolarizing block and antagonized a nondepolarizing block. HFL antagonized the phase 1 depolarizing block of decamethonium and Imbretil and potentiated the nondepolarizing dTC and phase 2 nondepolarizing block of decamethonium and Imbretil. These results cannot be explained adequately by the anti-pseudocholinesterase action of HFL. They can be explained by the nondepolarizing action of HFL. A nondepolarizing agent would be expected to potentiate the action of dTC, and a phase 2 nondepolarizing block of decamethonium or Imbretil, but antagonize a phase 1 depolarizing block of decamethonium and Imbretil. It is likely that HFL exerts a similar nondepolarizing effect on the phase 1 and phase 2 SCh block but that this effect is overshadowed by the anti-pseudocholinesterase or junctional membrane action of HFL.

In a previous paper\(^10\) it was pointed out that the production of a nondepolarizing block by SCh is not an occasional event requiring prolonged administration of large doses of SCh. SCh consistently produced a phase 2 nondepolarizing block after a cumulative dose of 3 mg./kg. In the present study decamethonium, Imbretil and HFL-SCh also produced a phase 2 nondepolarizing block. The administration of HFL reduced to 0.4–1 mg./kg. the amount of SCh required to produce a phase 2 nondepolarizing block. This may be explained in terms of the anti-pseudocholinesterase effect of HFL permitting an increased amount of SCh to reach the neuromuscular junction or by the junctional membrane effect of HFL.

Concerning the adverse effects of HFL, some studies report no effect on heart rate, cardiac rhythm, blood pressure, oropharyngeal secretions or bronchial tone.\(^2\),\(^3\),\(^5\) On the other hand, there are reports of tachycardia, hypotension, severe bronchospasm and cardiac arrhythmias.\(^7\),\(^8\) Bronchospasm, attributable to histamine release, was reported by Selvin and Howland,\(^7\) but was questioned by Foldes.\(^12\) Mostert\(^8\) however found that HFL produced bronchospasm in the rat and in man. He also reported that HFL-SCh produced tachycardia and cardiac arrhythmias in patients given halothane or trichlorethylene. He attributed the tachycardia and arrhythmias to a sympathomimetic action of HFL and concluded that there was no justification for the continued use of HFL in the clinical practice of anaesthesia. In the present study we regularly observed an increase in heart rate (as much as 100 per cent), an occasional increase in bronchial tone and oropharyngeal secretions, minor changes in blood pressure and, rarely, cardiac arrhythmias. While we cannot agree with Mostert that HFL should be abandoned, we do not feel that HFL-SCh is an ideal or even the best technique for producing muscle relaxation.
Summary

HFL had no effect on the twitch response in anesthetized man. The phase 1 depolarizing or phase 2 nondepolarizing block produced by SCV was increased by the injection of HFL. Edrophonium under similar circumstances increased the phase 1 depolarizing block, but antagonized the phase 2 nondepolarizing block. The phase 1 depolarizing block produced by decamethonium or Imbretil was antagonized by HFL while the phase 2 nondepolarizing block produced by decamethonium or Imbretil was potentiated by HFL. Edrophonium potentiated the phase 1 depolarizing block of decamethonium and Imbretil and antagonized the phase 2 nondepolarizing block of decamethonium and Imbretil. The dTC block was potentiated by HFL and antagonized by edrophonium. The results with edrophonium were attributed to inhibition of true cholinesterase. The effects of HFL were attributed to its junctional membrane, nondepolarizing and pseudocholinesterase inhibiting actions.

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References


Addendum

Fig. 1. Neuromuscular Junction: This represents a terminal nerve filament in cross section in the "gutter" at the neuromuscular junction. Synaptic vesicles probably contain acetylcholine (Ach), the normal chemical transmitter in inactive form. Release of vesicles is partially controlled by changes in membrane potential of the pre-synaptic membrane.
Fig. 2. **Normal Transmission:** The postsynaptic membrane has been enlarged almost to molecular dimensions. Ach is hydrolyzed by pseudocholinesterase, but the reaction of the true cholinesterase is so much faster that this overshadows the reaction in the plasma. The esteratic site and the receptor site are two separate areas. The formation of the complex Ach-receptor fraction causes sudden increase in ionic permeability of the membrane (and depolarization). The reaction ceases by reversibility of Ach and Receptor $\rightleftharpoons$ Ach-Receptor protein and rapid hydrolysis of Ach by the true cholinesterase. Use of true cholinesterase inhibitors slows or prevents the disappearance of Ach.

Fig. 3. **Nondepolarization Block:** d-Tubocurarine binds reversibly to the receptor protein but does not cause increased ionic permeability. d-Tubocurarine disappears by diffusion. During its occupation of the receptor site, Ach is blocked access and thus normal transmission ceases.

Fig. 4. **Depolarization Block—Phase I** is essentially the same as normal transmission by Ach depolarization. However, hydrolysis by pseudocholinesterase is almost as rapid as by true cholinesterase and most of the activity of succinylcholine disappears by hydrolysis in the plasma and only a small proportion by the true cholinesterase (shown by dotted activity line). Both types of cholinesterase inhibitors would be expected to increase the block. The different effects of hexafluorenium on succinylcholine, as compared with decamethonium and imbrelt, are attributable to other actions of hexafluorenium (see text).

Fig. 5. **Depolarization Block—Phase II** represents continued presence of large quantities of succinylcholine, either by prolonged administration or failure of the pseudocholinesterase mechanism (genetic, chemical, etc.). Membrane permeability is blocked (as in d-tubocurarine block). In addition, there is evidence that succinylcholine is present beneath the post-synaptic membrane. The pseudocholinesterase inhibitors would increase this block by preventing the destruction of succinylcholine molecules in the plasma. The true anticholinesterases would be expected to have no effect or increase this block. However, it is a clinical observation that the true anti-cholinesterases do reverse Phase II block. This effect is probably mediated by increased tension output by the recovering muscle fibers and not by the completely blocked fibers, as represented by this sketch.