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Decrease in Dose Requirement of d-*Tubocurarine by Volatile Anesthetics*

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Volatile anesthetics are known to decrease the requirements for neuromuscular blocking agents. To obtain a quantitative measure of the extent of this drug interaction, studies were performed on isolated guinea pig nerve–lumbrical muscle preparations exposed to methoxyflurane, halothane, isoflurane, diethyl ether, fluroxene, and enflurane in concentrations equal to MAC. From the relationship between indirect twitch height and *d*-tubocurarine concentration, the concentration depressing the twitch height by 50 per cent was determined. In the presence of MAC levels of anesthetic, the ED_{50} was decreased by the following fractional amounts: methoxyflurane, 0.311; halothane, 0.334; isoflurane, 0.335; diethyl ether, 0.462; fluroxene, 0.580; enflurane, 0.697. Comparison of the fractional decrease of *d*-tubocurarine dose requirement by an anesthetic at MAC and previously obtained values for the fractional depression of end-plate depolarization by an anesthetic at MAC showed that the more the anesthetic depresses depolarization, the smaller the *d*-tubocurarine dose requirement. Thus, clinically observed decreases in dose requirements may be explained by the effects of the anesthetics on chemosensitivity of the end-plate region. (Key words: Anesthetics, volatile: diethyl ether; enflurane; fluroxene; halothane; isoflurane; methoxyflurane. Muscle, skeletal: end-plate. Neuromuscular relaxants: *d*-tubocurarine.)

VOLATILE ANESTHETICS appear to interfere with neuromuscular transmission by depressing the postsynaptic response to the transmitter.[1–4] At concentrations equal to MAC, carbachol-induced depolarization of the end-plate is depressed 20 per cent by methoxyflurane, 19 per cent by halothane and isoflurane, 28 per cent by diethyl ether, 32 per cent by fluroxene, and 40 per cent by enflurane.[5] However, only with concentrations of anesthetic sufficient to block depolarization by 50 per cent will an anesthetic by itself depress the indirectly stimulated twitch response.[6] Therefore, at MAC, none of the above anesthetics would be expected to produce a neuromuscular block that could be measured clinically.

d-Tubocurarine also antagonizes carbachol-induced depolarization of the end-plate. While the nature of this antagonism is different (tubocurarine shows competitive kinetics, the anesthetic antagonism is insurmountable), tubocurarine, like the anesthetics, can produce considerable neuromuscular block before the twitch response is depressed. Specifically, the twitch response remains normal until 75–80 per cent of the acetylcholine receptors have been occluded.[7]

The above considerations suggest that concentrations of an anesthetic and *d*-tubocurarine that individually would have no effect on the indirectly stimulated twitch might, when combined, produce neuromuscular block. Indeed, it is well known that lesser doses of neuromuscular blocking agents are needed in the presence of volatile anesthetics. The present study was directed toward obtaining a quantitative measure of the extent of the drug interaction.

Methods

The experiments were carried out on isolated guinea pig nerve–lumbrical muscle preparations incubated in a 50-ml bath of Krebs' solution of the following composition (mM):sodium, 138; potassium, 5.9; calcium, 2.5; magnesium, 1.22; chloride, 1.23; dihydrogen phosphate, 1.2; sulfate, 1.22; bicarbonate, 25; plus glucose, 2.08 g/l. This solution was bubbled with oxygen, 95 per cent, and carbon dioxide, 5 per cent, and maintained at 37 C. The anesthetics studied were halothane, methoxyflurane, isoflurane, diethyl ether, fluroxene, and enflurane.

The nerve was placed in a tunnel electrode and stimulated every 10 sec with 0.3 msec pulses of twice maximal intensity. The resting muscle length was adjusted to give maximal developed tension. Initially, the preparation was equilibrated until the twitch response was stable, (about 30 min). The muscle bath was then changed to Krebs' solution which had been preequilibrated with one of the above-mentioned anesthetics in a concentration equal to MAC in man, and the anesthetic mixture was continuously bubbled through the bath. After the twitch response had again become stable, *d*-tubocurarine was added to the bath in graded doses to generate a twitch height-versus-*d*-tubocurarine concentration–response curve. Such curves were compared with corresponding control curves obtained in the absence of anesthetics. In the earlier experiments in this study, *d*-tubocurarine concentration–response curves were obtained for each muscle with and without anesthetics in random order. However, during analysis of the results, it was found that sensitivity to *d*-tubocurarine increased with time, presumably because the margin of safety of neuro-

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muscular transmission was falling. The latter phenomenon has been seen elsewhere and attributed to degeneration of the nerve ending following the denervation associated with isolation.[7] Therefore only results from the first dose–response curve obtained for each muscle were used.

Since volatile anesthetics may increase the twitch response to indirect stimulation,[6] twitch depression by *d*-tubocurarine had to be compared with two control twitch heights, that in the presence of an anesthetic and that in the absence of an anesthetic, to generate two slightly different measures of twitch depression (both reported in table 1). The effect of the anesthetic was expressed as a dose-ratio, calculated by dividing the *d*-tubocurarine concentration that blocked the twitch by 50 per cent (ED_{50}) in the presence of anesthetic by the control ED_{50}. (To obtain an objective estimate of these ED_{50} values, a sigmoid function, specifically twitch height = $1 - [d\text{-tubocurarine}]^P / ([d\text{-tubocurarine}]^P + ED_{50}^P)$, where the parameter P determines the slope of the curve, was fitted to the observations by an iterative least-squares technique analogous to that used previously in this laboratory.[5])

Finally, the decrease in *d*-tubocurarine dose requirement by anesthetics at MAC (1 − dose-ratio) was compared with the depression of depolarization by anesthetics at MAC. The latter values were obtained from a previous study.[5]

At the end of each experiment, first the *d*-tubocurarine and then the anesthetic were washed out to confirm that the twitch responses returned to the original levels. The concentrations of anesthetic in the gas mixture and in the bath solution were monitored by gas chromatography as previously described.[5]

Results

All anesthetics shifted the *d*-tubocurarine dose–response curves to the left. In the presence of an anesthetic agent at a concentration equal to MAC, a lower concentration of *d*-tubocurarine was needed to depress the twitch response (fig. 1). Whether the twitch depression following *d*-tubocurarine was measured as 50 per cent of the twitch response in the absence of an anesthetic or 50 per cent of the twitch response following administration of anesthesia made no statistical

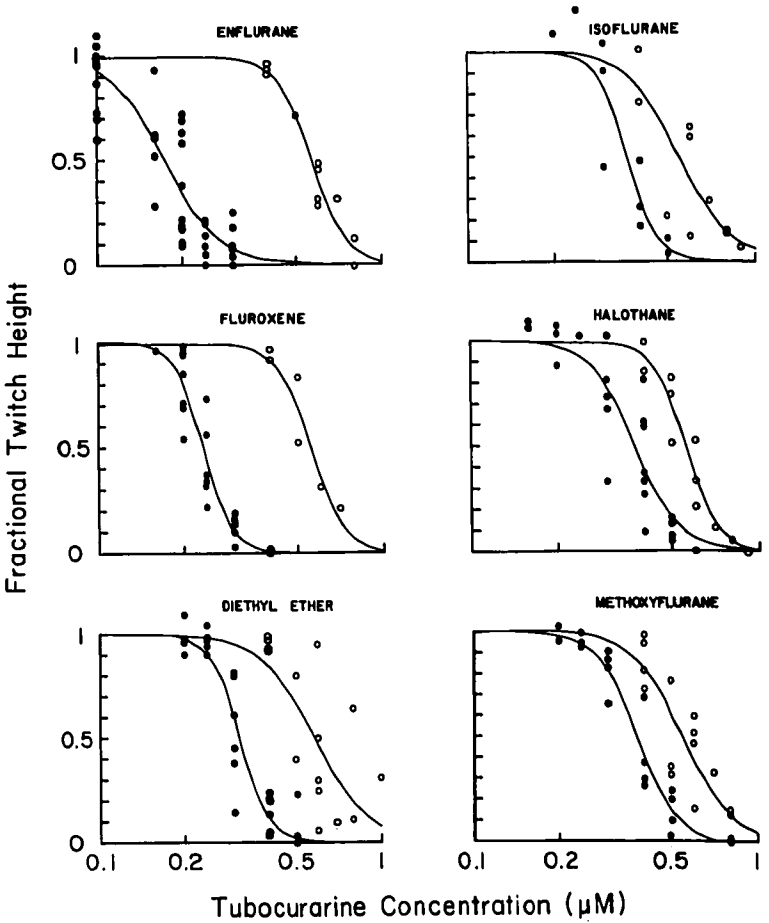

FIG. 1. Effects of anesthetics on tubocurarine dose–response curves. *Ordinates*: indirect twitch responses relative to control levels. *Abscissae*: *d*-tubocurarine concentrations (μM). *Open circles*: *d*-tubocurarine dose–response curves without anesthetic. *Closed circles*: *d*-tubocurarine dose–response curves at MAC anesthetic concentrations.

TABLE 1. *d*-Tubocurarine Dose Requirements

	End-plate Effect*	*d*-Tubocurarine ED_{50}, μM (Mean ± SE)†			B/A	Decrease in Tubocurarine Dose Requirement‡ (1 − B/A)
		Control A	With Anesthetic B	With Anesthetic C		
Methoxyflurane	20	0.546 ± 0.092 (5)	0.376 ± 0.041 (5)	0.376 ± 0.041 (5)	0.689	0.311
Halothane	19	0.557 ± 0.042 (3)	0.371 ± 0.070 (7)	0.351 ± 0.068 (7)	0.666	0.334
Isoflurane	19	0.544 ± 0.120 (3)	0.362 ± 0.054 (3)	0.345 ± 0.046 (3)	0.665	0.335
Diethyl ether	28	0.580 ± 0.162 (5)	0.312 ± 0.039 (9)	0.308 ± 0.306 (9)	0.538	0.462
Fluroxene	32	0.557 ± 0.044 (2)	0.234 ± 0.021 (7)	0.234 ± 0.021 (7)	0.420	0.580
Enflurane	40	0.571 ± 0.033 (4)	0.173 ± 0.044 (11)	0.166 ± 0.047 (11)	0.303	0.697

* Expressed as percentage of depression of carbachol-induced end-plate depolarization produced at MAC (depression of 50 per cent is necessary before any depression of the indirect twitch response is seen). From previous study.[5]

† Columns A–C represent concentrations of *d*-tubocurarine from three types of *d*-tubocurarine concentration–response curves in isolated nerve–lumbrical muscle preparations. Three classes of response are involved. When the muscle is first set up there is a reference level of twitch height (R_1). If anesthetic is given this "control" level may shift to a new value (R_2). Finally, in the presence of graded concentrations of *d*-tubocurarine, a series of reduced twitch heights (T_1) is obtained. Column A represents the results from preparations not exposed to an anesthetic; the ED_{50} values are obtained from the responses T_1/R_1. Columns B and C give results from preparations exposed to an anesthetic. In column B, R_1 was taken as the reference response so ED_{50} values were calculated from the ratios T_1/R_1. In column C, the ratio T_1/R_2 was used. Numbers of preparations are given in parentheses.

‡ Effect of anesthetic on *d*-tubocurarine dose requirement, calculated by dividing ED_{50} in the presence of anesthetic by control ED_{50}. For this purpose the values in column B were used. For example, in the presence of halothane at MAC, 0.371/0.557 = 0.666 times the dose of *d*-tubocurarine must be used to block the twitch response. Thus, the *decrease* in *d*-tubocurarine dose requirement becomes 1 − 0.666 = 0.334.

difference in the ED_{50} values for *d*-tubocurarine (table 1). There is a direct relationship between fractional decreases in *d*-tubocurarine dose requirement by anesthetics at MAC and fractional depression of depolarization by anesthetics at MAC (fig. 2). That is, the more the anesthetic depresses end-plate depolarization, the smaller the dose requirement of *d*-tubocurarine.

Discussion

It is well known that patients receiving a volatile anesthetic require less *d*-tubocurarine to block neuromuscular transmission than do patients receiving balanced anesthesia. Studies measuring the additive effects of *d*-tubocurarine and ether,[8] methoxyflurane,[9] halothane,[10,11] or enflurane[12,13] in patients or volunteers have shown that with anesthetic concentrations insufficient to decrease the indirect twitch response, the blocking effect of *d*-tubocurarine was greater than when a volatile anesthetic was not used. Since the combined effect of antagonist plus methoxyflurane[9] or halothane[11] was not changed by ulnar nerve block, the enhancement of neuromuscular block caused by these agents must be attributed to a peripheral rather than a central nervous system event. At the neuromuscular junction, volatile anesthetics depress carbachol-induced depolarization in a dose-related manner.[5] Only when the anesthetic concentration is sufficient to depress depolarization by 50 per cent, however, does the indirect twitch response begin to fail.[6] (The concentrations of anesthetic needed to depress depolarization by 50 per cent range from 1.25–1.57 MAC with enflurane to 2.83–3.67 MAC with halothane.[5]) Nevertheless, although the anesthetic concentration may be too low to alter reaction to nerve stimulation, end-plate sensitivity is decreased and the addition of *d*-tubocurarine would have a greater blocking effect than if the volatile anesthetic were omitted.

An attempt to compare the relative effects of various anesthetics on *d*-tubocurarine dose requirement by comparing the studies in the literature proves difficult. Most investigators examined only one agent,[8–11] and techniques were not alike. Another study[14] compared effects of unequal concentrations, *i.e.*, halothane, 1.3 MAC, diethyl ether, 2.1–2.6 MAC, and methoxyflurane, 3.1–6.2 MAC. In yet other reports,[12,15] *d*-tubocurarine dose requirements were

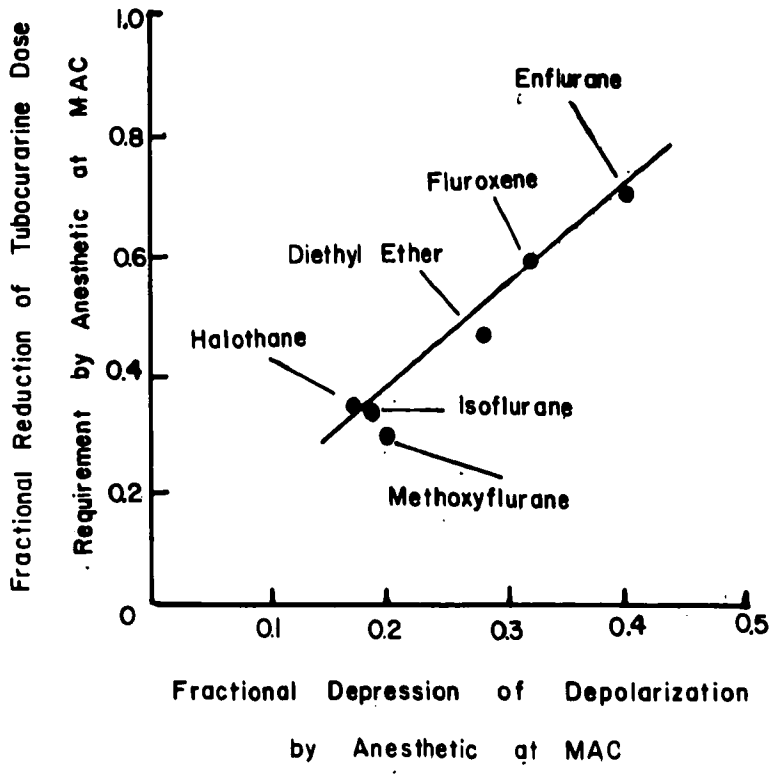

FIG. 2. Relation of reduction of *d*-tubocurarine dose requirement to ability of anesthetics to block end-plate depolarization. *Ordinates*: 1 − [(*d*-tubocurarine ED_{50} in the presence of MAC anesthetic)/(control *d*-tubocurarine ED_{50})]. *Abscissae*: 1 − [(depolarization in presence of MAC anesthetic)/(control end-plate depolarization produced by carbachol)], calculated from previous results.[5]

compared among anesthetic agents, but the *d*-tubocurarine dosage in the absence of a volatile agent was not measured. Nevertheless, all these studies indicate that volatile anesthetics can decrease the dose of *d*-tubocurarine needed to cause neuromuscular block. Furthermore, such a decrease in dose requirement appears to be least with halothane, intermediate with diethyl ether, and greatest with enflurane. The present study confirms this impression and adds quantitative information. Thus, at concentrations of anesthetic equal to MAC, there is no depression of the twitch response, but the concentration of *d*-tubocurarine needed to depress the twitch by 50 per cent must be decreased by a third with methoxyflurane, halothane, and isoflurane, by approximately half with diethyl ether and fluroxene, and by two thirds with enflurane. Of the agents examined in the present series, only isoflurane yields results that do not fit with those of human studies. In the latter, isoflurane decreased the *d*-tubocurarine dose requirement more than did halothane[15] and, in fact, behaved like enflurane.[12] Experiments on rat phrenic nerve–diaphragm, however, agree with the present study. As has already been suggested,[16] the greater potentiation of *d*-tubocurarine by isoflurane *in vivo* may be the result of a threefold increase in muscle blood flow associated with isoflurane, which thus enables a greater fraction of *d*-tubocurarine to reach the neuromuscular junction.

The extent of the interaction of volatile anesthetics and tubocurarine may be explained by events at the neuromuscular junction. As table 1 shows, the more the anesthetic blocks end-plate depolarization, the smaller the *d*-tubocurarine requirement. The excellent fit seen in figure 2 further indicates that the effect of anesthetics on the chemosensitivity of the end-plate region appears to be the basis of the observed drug interaction. At MAC the end-plate effect varies from 19 to 40 per cent depression, and thus explains the variation in *d*-tubocurarine requirements with different anesthetics at this concentration. With increasing anesthetic concentrations, block of depolarization becomes greater.[5] The dose of *d*-tubocurarine needed to block the neuromuscular junction, therefore, will decrease as the concentration of anesthetic increases.

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