

Effects of Volatile Anesthetics on Directly and Indirectly Stimulated Skeletal Muscle

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Isolated guinea pig nerve-lumbrical muscle preparations were exposed to halothane, methoxyflurane, isoflurane, enflurane, fluoro-xene, and diethyl ether. The temporal courses of the effects on indirectly and directly elicited twitch responses were determined over a range of concentrations for each agent. When the anesthetics were compared at concentrations equivalent in terms of minimum alveolar concentration (MAC), a spectrum was observed in which halothane, methoxyflurane and isoflurane depressed the indirect twitch response at 3.5-5 MAC and the direct twitch response at 8-10 MAC. Diethyl ether and fluoro-xene depressed the indirect twitch response at 2-3.5 MAC and the direct twitch response at 3-6 MAC. Enflurane depressed the indirect response at 1.5-2.5 MAC and the direct response at 6-8 MAC. When the anesthetics were compared at concentrations equivalent in terms of their abilities to depress end-plate depolarization, however, all anesthetics were equipotent. Depression of the indirect twitch response occurred only when anesthetic concentrations were great enough to depress depolarization by 50 per cent. (Key words: Anesthetics, volatile: diethyl ether; enflurane; fluoro-xene; halothane; isoflurane; methoxyflurane. Muscle, skeletal: contraction. Neuromuscular transmission.)

VOLATILE ANESTHETICS have long been known to interfere with the responses of striated muscle to both direct and indirect stimulation.¹ Although the extents of these effects have been shown to depend on the agent studied,²⁻⁴ there is still considerable variation in the published results. For example, Pollard and Millar⁴ observed complete block of indirect twitch response with halothane, 5.6 per cent, while Sabawala and Dillon³ obtained complete block with 4 per cent. Results may reflect differences in temperature (32 *vs.* 37 C) or different durations of exposure to the anesthetic. Pollard and Millar restricted the time to 20 min, while Sabawala and Dillon stopped the anesthetic administration only when there was no response to indirect stimulation. Previous work⁵⁻⁹ has shown that the principal cellular action of volatile anesthetics appears to be related to their ability to depress the post-synaptic response to the transmitter. In particular, reported values of anesthetic potencies (minimal alveolar concentrations; MAC) have correlated well with

abilities to depress drug-induced depolarization of the end-plate region of guinea pig lumbrical muscles.⁹

The purpose of the present experiments was to compare actions of anesthetics on end-plate depolarization with their actions on the response of skeletal muscle to indirect stimulation. Further, the muscle responses to indirect and direct stimulation at several concentrations of each anesthetic were compared. The experiments were done *in vitro* to facilitate accurate control of drug concentrations. Particular attention was also given to kinetics of onset of action because of a prior impression that variations in exposure to an anesthetic before making observations might have been contributing to apparent discrepancies reported in the medical literature.

Methods

The experiments were carried out on 106 isolated guinea pig nerve-muscle preparations incubated in 50 ml Krebs' solution of the following composition (mM): sodium, 138; potassium, 5.9; calcium, 2.5; magnesium, 1.22; chloride, 123; dihydrogen phosphate, 1.2; sulfate, 1.22; bicarbonate, 25; plus glucose, 20.8 g/l. The solution was bubbled with 95 per cent oxygen and 5 per cent carbon dioxide and kept at 36-37 C. The anesthetics studied were enflurane, fluoro-xene, diethyl ether, methoxyflurane, isoflurane, and halothane.

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. The twitch response to direct stimulation was also examined. To this end, supramaximal shocks of 0.5-msec duration were applied every 10 sec through platinum rings about 3 mm in diameter, located around each end of the muscle. Such field stimulation requires intense currents (about 200 mamp), and therefore the possibility of accumulation of electrolytic products arises. To decrease this effect, a switching circuit was used such that stimulation polarity was reversed for alternate pulses. The strong stimuli used for this direct stimulation are well above threshold for activation of nerve fibers running through the muscle, so a blocking dose of *d*-tubocurarine was added to eliminate indirect activation of the muscle (1 μ M *d*-tubocurarine produces neuromuscular block in the guinea pig lumbrical muscle: 4 μ M was used here to ensure complete block).

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When the twitch responses to indirect or direct stimulation had reached a steady state, the muscle was bathed in Krebs' solution which had been pre-equilibrated with the concentration of the anesthetic to be studied. This anesthetic concentration was bubbled through the muscle bath until the twitch response had not changed for 20–30 min. The anesthetic was then washed out until the twitch response had returned to its control height. At lower concentrations of anesthetics, responses to indirect stimulation were followed in the same preparation by responses to direct stimulation at the same anesthetic concentrations. At high anesthetic concentrations, where time allowed, two direct responses at different concentrations were obtained. To facilitate summarization of such experiments, two specific measures were used: 1) the highest twitch response obtained—the peak response; 2) the final stable twitch response—the steady-state response.

Both the peak effect of the twitch response and the steady-state level obtained with indirect and direct stimulation were recorded and compared with each other and with previously determined activities of the anesthetics, such as production of anesthesia or depression of depolarization.

Because the effects of volatile anesthetics show a steep dose–response curve, anesthetic concentrations were carefully controlled and monitored as previously described.⁹ Every 30 min, the bath mixture was replaced with fresh Krebs' solution pre-equilibrated with the appropriate anesthetic concentration.

Results

The onsets of action of all anesthetics on the twitch response showed a characteristic general pattern. At low concentrations, twitch height increased slightly, if at all, and did not fade with time. At higher concentrations, twitch height increased to a peak at about 10 min and faded slowly over the next one to four hours to reach a steady state. At still higher concentrations, the initial stimulatory response was rapidly superseded by twitch depression. Figure 1 shows representative examples obtained with halothane and diethyl ether. Because of this temporal lability of the twitch response, both peak and steady-state values are presented in the following discussion (figs. 2–7).

Although the responses seen with any given anesthetic concentration showed considerable variation, the anesthetics could be divided clearly into two groups, those that increased twitch height to as much as 2.5 times control (halothane, methoxyflurane, isoflurane, and enflurane, figs. 2–5), and those that caused minimal stimulation (diethyl ether and flu-

roxene, figs. 6 and 7). The first group of four anesthetics could not be further divided into subclasses on the basis of their abilities to increase the twitch response following either indirect or direct stimulation. Examination of the remaining two anesthetics left the impression that fluroxene may produce more stimulation than diethyl ether, but the effect was not striking. With all anesthetics, the increased twitch responses to indirect and direct stimulation went hand-in-hand until anesthetic concentrations were great enough to cause depression of the indirectly stimulated twitch.

Like the degree of stimulation, the degree of twitch depression depended on the anesthetic agent, the concentration, and the duration of exposure of the muscle (fig 1). In addition, there was a dependence on the extent of the preceding stimulation. At low concentrations, maximum twitch response was achieved in minutes and was maintained. As the anesthetic concentrations were increased, there were further increases in the peak twitch response, but this initial stimulation of the twitch response was not maintained. The times to reach steady state increased with increasing concentrations (the greater the depression, the longer the time) until the depressant effect became the dominant factor in the twitch response. Again, the agents could be divided into groups. Halothane, methoxyflurane, and isoflurane depressed the indirect twitch response at 3.5–5 MAC and the direct twitch response at 8–10 MAC. Diethyl ether and fluroxene depressed the indirect twitch response at 2–3.5 MAC and the direct twitch response at 3–6 MAC. The effects of enflurane differed from those of both groups, in that the indirect response was depressed at 1.5–2.5 MAC and the direct response was depressed at 6–8 MAC.

The indirect twitch response was compared with the previously reported⁹ ability to depress depolarization of the end-plate region. This was done by calculating the extent by which end-plate depolarization would be depressed by concentrations producing twitch depression in the present study. These values, plotted in figure 8, showed the anesthetics to be strikingly equipotent. The twitch response did not fail until concentrations that depressed depolarization by 50 per cent were reached, and complete block was seen at levels that depressed depolarization by 70 per cent.

All the effects of the anesthetics were reversible.

Discussion

The effects of volatile anesthetics on both indirect and direct twitch responses have been studied for more than 60 years, with considerable variation in the results reported.^{1–4} Much of the discrepancy may

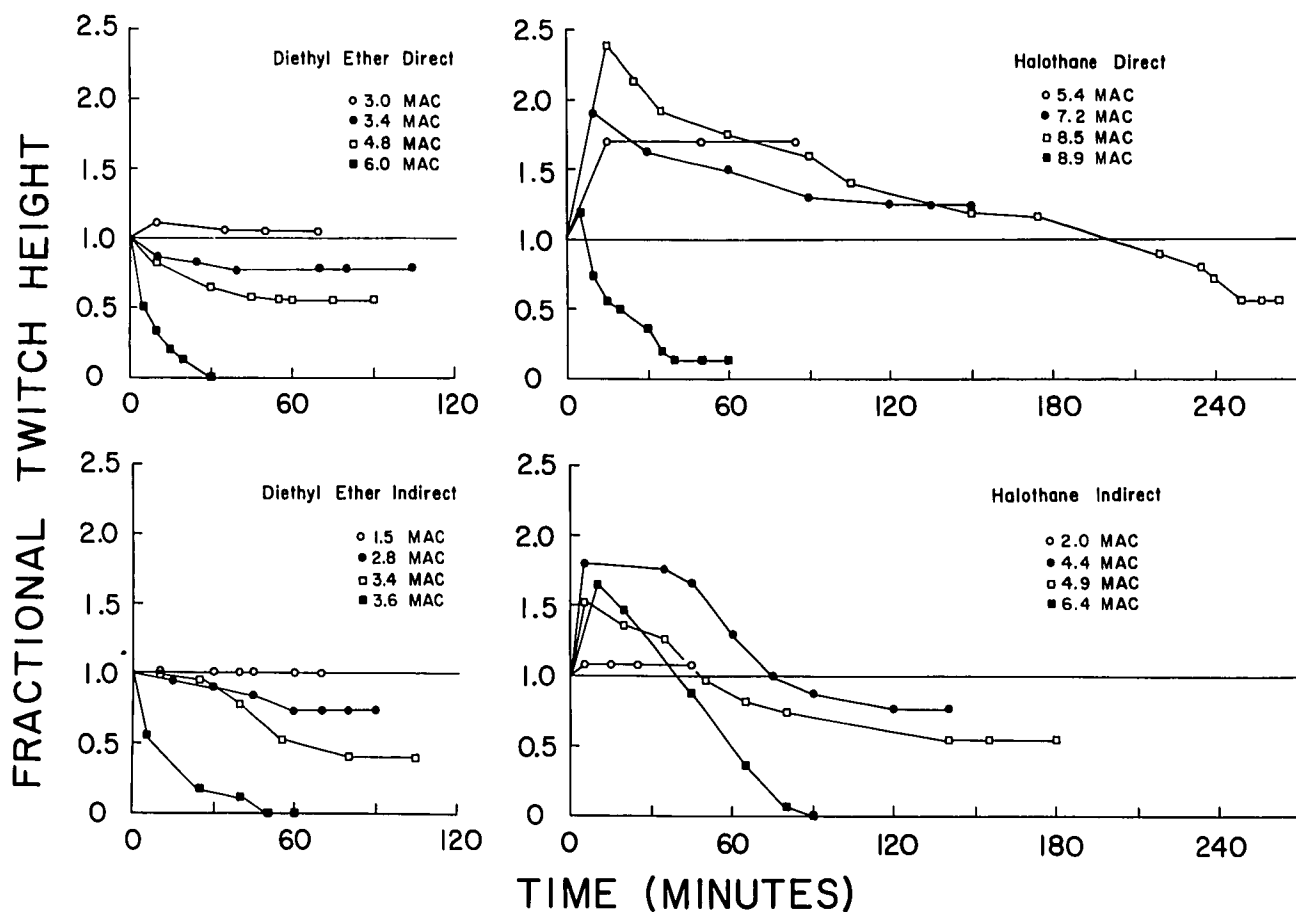


FIG. 1. Examples of kinetics of onset of anesthetic action. *Ordinates*: Twitch heights relative to control value. *Abscissae*: Times in minutes. *Upper panels*: Responses to direct stimulation. *Lower panels*: Responses to indirect stimulation. *Left panels*: Diethyl ether. *Right panels*: Halothane. Concentrations in MAC as indicated in each panel. Each curve represents the values obtained during onset of action of the anesthetic in an isolated muscle.

be explained by the great temporal lability of the responses of muscles to an anesthetic. For example, the direct twitch response to 8.5 MAC halothane took four hours to reach a steady state, while the response to 8.9 MAC halothane took 40 min. Certainly a study that used a 20-min "equilibration" time could differ from one with a 40-min time, and this, in turn, would not always represent a steady-state situation.

In this study, the guinea pig nerve-muscle preparation proved to be a hardy preparation that could withstand many hours of high concentrations of anesthetics without irreversible changes. Thus, we were able both to study the effects of high concentrations of anesthetics and to wait until a steady state was obtained. Peak values were examined additionally because the peak was a clearly defined point that could reflect transient behavior.

The results of this study may be discussed from either the mechanistic or clinical point of view. We discuss each in turn.

MECHANISTIC ANALYSIS

The first step in the analysis is to examine the twitch response qualitatively. Figures 1-7 show clearly that there are actions both to augment and to depress twitch responses. Consider first augmentation. The similar degrees of augmentation seen with directly and indirectly elicited twitches indicate an effect directly on the muscle, *i.e.*, on the excitation-contraction coupling or contractile mechanism. The possibility of repetitive firing in the nerve ending as a cause for the increased twitch response can be ruled out because stimulation was obtained even in the presence of large doses of *d*-tubocurarine.¹⁰

General anesthetics have also been shown to increase the tension of caffeine-induced contractures.¹¹ The increased tensions developed by muscles in the presence of concentrations equal to MAC, as compared with control tensions, show halothane and methoxyflurane to be most active and fluroxene and

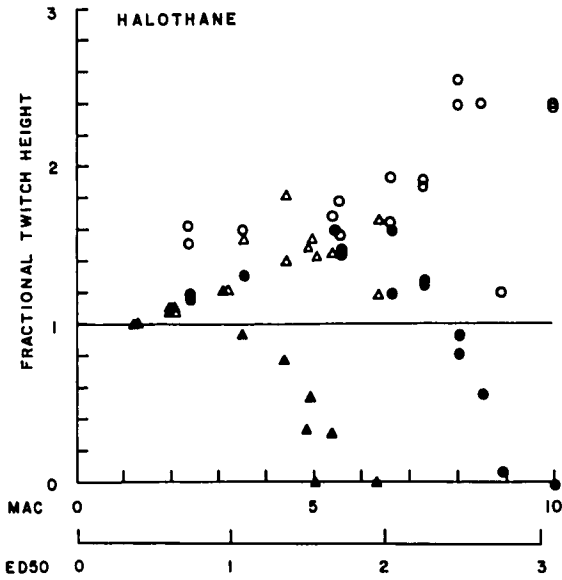


FIG. 2. Dose-response relationships for halothane. *Ordinates*: Twitch heights relative to control values. *Abscissae*: Anesthetic concentrations in units of MAC (*upper scale*) or in units of ED₅₀ for depression of end-plate depolarization (*lower scale*; see text). *Open symbols*: Peak responses. *Closed symbols*: Steady-state responses. *Triangles*: Indirect stimulation. *Circles*: Direct stimulation. Each symbol represents the result from a separate preparation. Thirteen muscles received indirect stimulation; 16, direct.

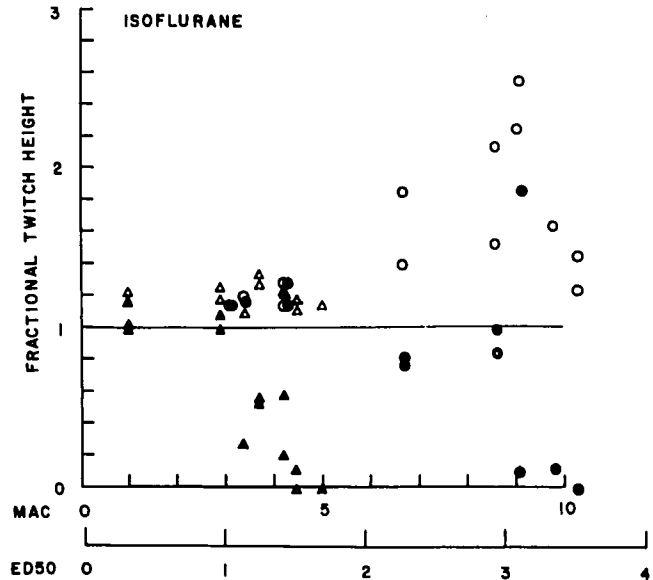


FIG. 4. Dose-response relationships for isoflurane. Plot as in figure 2. Twelve muscles received indirect stimulation; 13, direct.

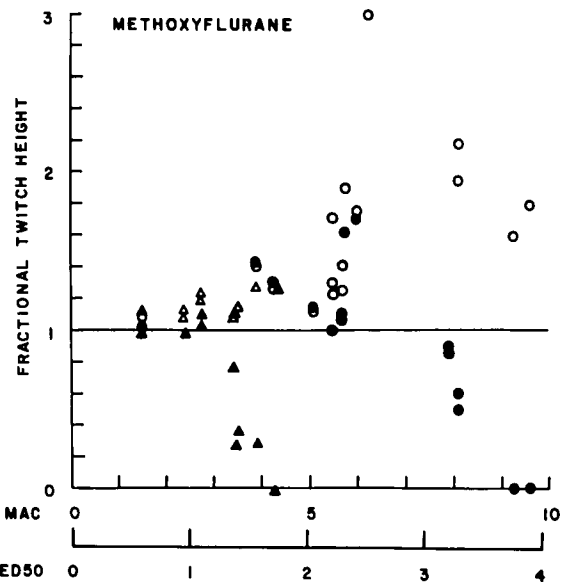


FIG. 3. Dose-response relationships for methoxyflurane. Plot as in figure 2. Ten muscles received indirect stimulation; 18, direct.

diethyl ether to be least active. The ranking of potencies of the effects of the anesthetics on caffeine-induced contractures is in excellent agreement with the potencies observed in the present experiments. This, again, supports the muscle cell as the site of

action of the stimulatory effect of the anesthetics studied.

Now consider depression of twitch response. The much greater sensitivity of the indirect response indicates that there were at least two anesthetic actions depressing the twitch in the present experiments. One appeared at low concentrations and affected something activated only during indirect stimulation. This may be explained by the previous observation⁹ that volatile anesthetics depress depolarization. As figure 8 shows, when depression of depolarization is plotted against depression of twitch height, all the anesthetics fall along the same curve. Depression of the direct twitch response at higher concentrations suggests a site of action peripheral to the neuromuscular junction, *i.e.*, on excitation-contraction coupling or on the contractile mechanism.

Thus, peripheral actions of anesthetics alter the twitch response in three ways. First is the depression of depolarization of the end-plate region. Here, all the anesthetics behaved similarly. Consideration of the other two effects, stimulation followed by depression of the muscle cell distal to the end-plate, divides the anesthetics into two categories, those that caused minimal stimulation (diethyl ether and fluroxene) and those that caused large degrees of stimulation (halothane, methoxyflurane, isoflurane, and enflurane). Those anesthetics that cause the greatest stimulation require the highest concentrations to block muscle contraction.

When peripheral anesthetic effects are now compared with CNS potencies in terms of MAC equiva-

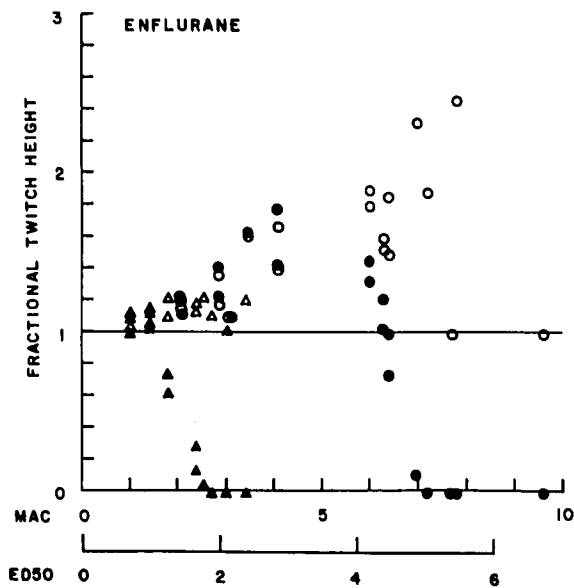


FIG. 5. Dose-response relationship for enflurane. Plot as in figure 2. Twelve muscles received indirect stimulation; 19, direct.

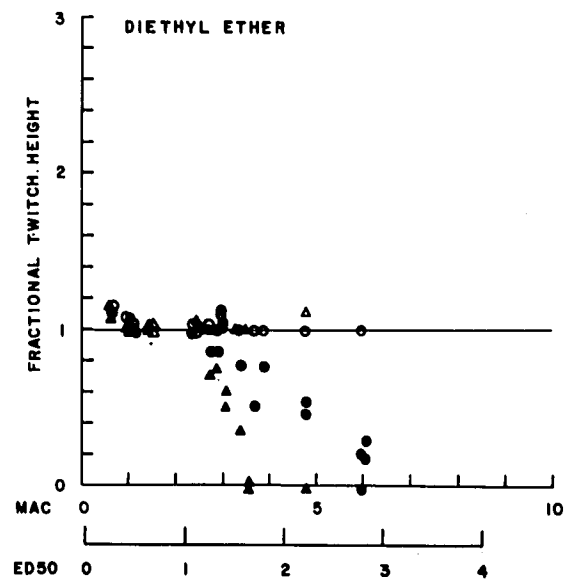


FIG. 6. Dose-response relationships for diethyl ether. Plot as in figure 2. Thirteen muscles received indirect stimulation; 18, direct.

lents (figs. 2-7) a third anesthetic category must be considered. Specifically, diethyl ether and fluroxene still fall together, depressing the indirect twitch response in the range of 2-3.5 MAC and the direct twitch response at 3-6 MAC. Similarly, halothane, methoxyflurane, and isoflurane depressed the indirect response at 3.5-5 MAC and the direct response at 8-10 MAC. Enflurane, however, no longer resembles the latter group. Indeed, indirect stimulation was depressed at lower anesthetic concentrations than with either group (1.5-2.5 MAC), while the direct response was depressed at concentrations between the two groups (6-8 MAC). The aberrant behavior of enflurane must be a central effect, since it became apparent only when peripheral and central effects were compared. Such behavior may, in fact, reflect simply the well-known ability of enflurane to stimulate the CNS. With enflurane, greater concentrations would be needed to reach the end point of the MAC assay, *i.e.*, the MAC for enflurane would be higher than the value that would reflect only peripheral depression.

That we are dealing simply with a problem of scaling can be illustrated with a change in scale. Rather than use as our frame of reference MAC, which can reflect a balance between excitatory and depressant cellular events (see fig. 9), another ED_{50} may be chosen with a view to getting one that reflects only a single cellular event. The ED_{50} values for depression of depolarization reported previously⁹ will serve the purpose. These ED_{50} values have been used to construct the lower concentration scales in figures 2-7. Measured

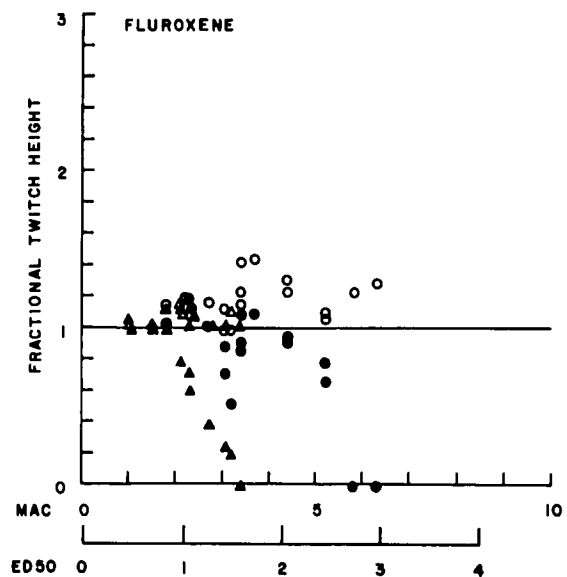


FIG. 7. Dose-response relationships for fluroxene. Plot as in figure 2. Eleven muscles received indirect stimulation; 18, direct.

against this new scale, all agents are equipotent in depressing the indirect twitch; all do so between ED_{50} values of 1 and 2 units.

In summary, the actions of the anesthetics may be explained as follows. When depression of indirect twitch response is considered in terms of depression of depolarization, all anesthetics are equipotent. On the other hand, when depression of indirect twitch response is considered in terms of MAC, a CNS effect becomes apparent. At one extreme is enflurane, with

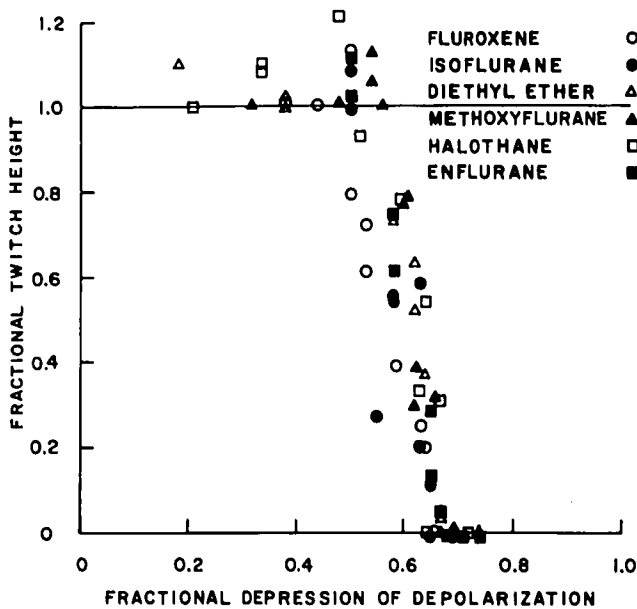


FIG. 8. Comparison of anesthetic effects on fractional twitch height and depression of depolarization. *Ordinates*: Twitch heights as fraction of control values. *Abscissae*: Corresponding degrees of depression of end-plate depolarization previously obtained.⁹ All anesthetics behave similarly.

its CNS stimulation and therefore relatively high MAC. At the other extreme are halothane, methoxyflurane and isoflurane, with no known CNS stimulatory effects. The difference between the latter three agents and diethyl ether and fluroxene implies that the latter two agents have latent CNS stimulating activity. Indeed, this has been reported to be the case for diethyl ether.¹²

When directly stimulated twitch responses are examined in terms of MAC, we again see three groups of anesthetics. However, there is no longer homogeneity when twitch response is compared with depression of depolarization. In other words, another factor must be considered. The obvious candidate is the stimulatory effect on the muscle cell.

When the steady-state effects of direct stimulation are examined, the twitch response can be viewed as a balance between the stimulatory and depressant effects of the anesthetic used. Thus, agents that cause the most stimulation require the highest concentrations to produce block. With diethyl ether, which causes little or no augmentation of the twitch response, direct and indirect stimulation are blocked at similar concentrations. This "tug-of-war" between stimulation and depression can be seen even with the results of a single agent. At 3.5 MAC fluroxene, stimulation was 145 per cent of control and, although this was followed by considerable depression, the steady-state response was still above the control level (fig. 7).

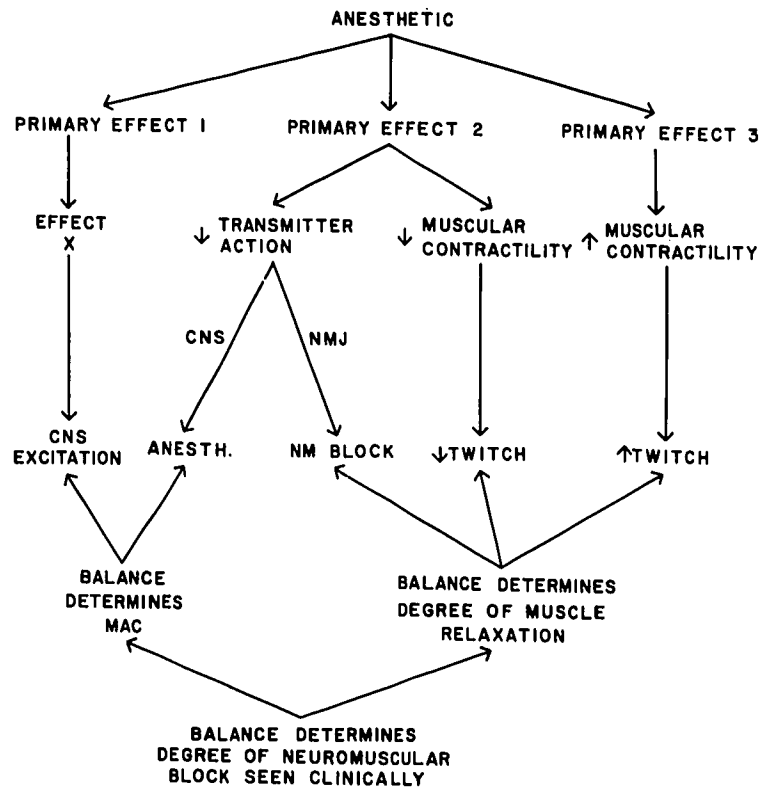
The above analysis is summarized in figure 9. Enflurane, in particular, but also diethyl ether and fluroxene, have been assigned CNS stimulating activity. It is interesting to review earlier results in this light. In a previous paper,⁹ the ranking of the anesthetics on the MAC scale was compared with that on a concentration scale derived from their potencies in depressing end-plate depolarization. The values are given in figure 10, where the broken line is the least-square regression and has a slope of 1.03. If only halothane, methoxyflurane and isoflurane show relatively pure anesthetic effects uncomplicated by CNS stimulation, then it would be more appropriate to use a regression involving only these three agents. This is represented by the solid line in figure 10, a regression with unit slope fitted to the three reference agents. In this modified form the diagram of figure 10 summarizes the effects of intrinsic CNS stimulation by showing diethyl ether and fluroxene slightly above the solid regression line and enflurane higher still, positions reflecting MAC values inflated by the necessity to give enough anesthetic to counteract the CNS stimulation.

Before leaving this discussion of a conceptual model, a few words of caution are in order. Since the picture is intrinsically complex, the proposed model has been presented in as simple a form as possible. Thus, for example, anesthetics have been divided into three groups with the implication that enflurane, diethyl ether, and fluroxene exert CNS stimulatory actions, whereas, halothane, methoxyflurane, and isoflurane are completely devoid of stimulatory activity. Such an all-or-none distinction is probably an oversimplification. With regard to ability to exert any of the primary effects of figure 9, anesthetics are more likely to lie in a spectrum rather than in discrete groups. Indeed, Bianchi's figure 8-5 suggests such a spectral distribution.¹¹ While halothane, methoxyflurane, isoflurane, and enflurane clearly differ from diethyl ether and fluroxene, quantitatively there is considerable variation in the extents of the differences. Isoflurane does not differ as much as halothane and methoxyflurane.

CLINICAL IMPLICATIONS

Consideration of figure 8 shows that with all anesthetic agents end-plate depolarization must be depressed by 50 per cent before the twitch response begins to fail. Deep neuromuscular block is seen only when end-plate depolarization is depressed by 70 per cent. In other words, considerable neuromuscular block is present before a block is detectable clinically. Administration of an anesthetic at 1 MAC will produce 20-40 per cent depression of depolarization.

FIG. 9. An anesthetic can exert any or all of three primary actions, which are presumably at the molecular or physical-chemical level. At the next, or cellular, level, these primary effects become expressed in four ways. First, there is some cellular event in the CNS of undefined nature that could be either stimulation of an excitatory process or depression of an inhibitory process (effect x in the diagram). Second, there is interference with the action of transmitters both in the CNS and in the periphery. Third, there is a direct depression of muscle contractility in the periphery. Finally, there is a direct effect to increase muscle contractility. At the level of the whole organ, the interaction of the cellular effects is expressed. In the CNS, "effect x" and depression of transmitter action lead to excitation and depression of consciousness, respectively, with the latter effect predominant among the clinically used agents. In fact, it is only with enflurane (and possibly ether and fluroxene, see below), that excitatory effects are really noticeable. Similarly, in the nerve-muscle system, the end effect observed reflects a balance of forces, three in this case. Depression of transmitter action and direct depression of muscle contractility induce muscular relaxation while the excitatory effect on muscle opposes it. Finally, the whole organism may be considered. At this level it is the degree of muscle relaxation produced by an anesthetic concentration that is of interest. As the diagram indicates, all of the basic factors interact to produce the effect observed.



In other words, twitch height will not be decreased. It is important to note, however, that this does not imply that there is no clinical effect. The fact that end-plate sensitivity has been decreased will be expressed clinically as a decreased dosage requirement for neuromuscular blocking agents.

The differences among agents are also of considerable clinical interest. Halothane, methoxyflurane, and isoflurane provide a good starting point. At clinical anesthetic concentrations, figures 2, 3, and 4 indicate that there would be no depression of the indirectly elicited twitch, *i.e.*, there would be no muscle relaxation. (In fact, the predominant observation may be an increased twitch response, a reflection of direct stimulation of the muscle cell.) On the other hand, diethyl ether, fluroxene, and enflurane, because of their abilities to produce CNS stimulation, have increased MAC values. Clinically, this means that they are used at higher absolute concentrations than would otherwise be the case. With diethyl ether and fluroxene the increases in concentration are such that neuromuscular block may be seen during deep anesthesia. In turn, with enflurane, the effect is so marked that neuromuscular block might appear even during light anesthesia.

It is interesting to compare these potencies with what is seen in man. Enflurane has been shown to

require much less supplemental relaxation than halothane.¹³ One's general clinical impression is that ether and fluroxene, too, are synergistic with α -tubocurarine (although solid quantitative evidence in man has surprisingly not been reported). On the other hand,

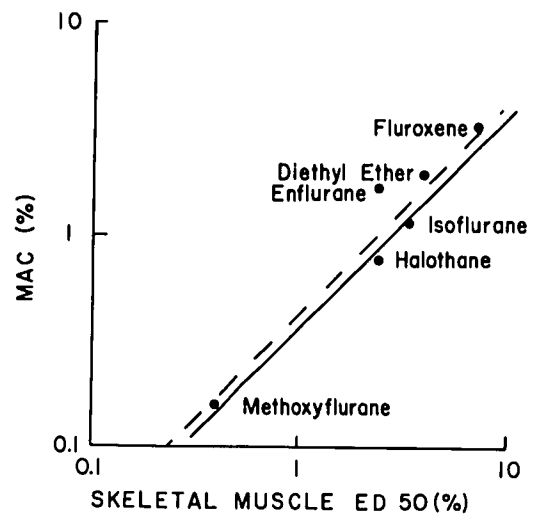


FIG. 10. Comparison of anesthetics. Ordinate: MAC. Abscissae: ED₅₀ for suppression of end-plate depolarization. Data from previous study.⁹ Broken line: Least-squares fit to all six values. Solid line: Fit with unit slope to methoxyflurane, halothane, and isoflurane. See text.

methoxyflurane has not been found to demonstrate much by way of relaxant activity. Although not in wide clinical use, isoflurane has been studied and its use found to be associated with a considerably lesser requirement for *d*-tubocurarine.¹⁴ However, this apparent exception seems to be related not to an effect at the neuromuscular junction but rather to a circulatory action or the like, since the effect was not seen in a controlled *in-vitro* situation.¹⁵ Thus, the relative activities seen in the present study parallel very satisfactorily what is seen clinically.

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