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Difference of train-of-four fade induced by nondepolarizing neuromuscular blocking drugs: a theoretical consideration on the underlying mechanisms

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Abstract: Nondepolarizing neuromuscular blocking drugs induce train-of-four (TOF) fade, i.e., the reduction of the fourth to the first twitch height in a train under TOF stimulation. It has been observed that the degree of TOF fade varies with the drug used and is inversely correlated with the potencies of the drug. In this study, the cause of difference of TOF fade was considered using a dynamic model. The model was based on the following assumptions: (1) Twitch response is evoked by the binding of acetylcholine (ACh) molecules to the postsynaptic nicotinic receptors in a neuromuscular junction, (2) time-dependent ACh mobilization in a motor nerve terminal results in less ACh output at the fourth stimulus in a train than at the first stimulus, (3) the drugs compete with ACh for the postsynaptic receptors and inhibit the receptor-binding of ACh, and (4) the drugs have various affinities for the receptors. This study suggested that the difference of affinities of the drugs for postsynaptic ACh receptors may cause the difference of TOF fade.

Key words: Train-of-four fade, Nondepolarizing neuromuscular blocking drugs, Acetylcholine, Mobilization, Model

Introduction

Train-of-four (TOF) stimulation at frequencies lower than 4.0 Hz is used to monitor neuromuscular function [1–7]. The four successive twitch height responses (T1, T2, T3, and T4) evoked by the stimulation have similar height in the absence of relaxants. Nondepolarizing neuromuscular blocking drugs, however, induce fade in the series of four twitch heights as well as the depression of T1. The TOF ratio (T4/T1) at a certain degree of T1 depression varies depending on the drugs [3–5]. It has been considered that the difference of TOF fade among the drugs is due to the presynaptic action of the drugs [8–10]. Recently, it has been suggested that the TOF fade has no relation with the presynaptic action [11]. The mechanisms underlying the various TOF fade have not yet been fully elucidated. Careful survey of the reported data [3–5], however, indicated the existence of regularity: the degree of TOF fade is almost inversely related to the potencies of the drugs. TOF fade is a clinical index of the depth of neuromuscular blockade produced by the drugs. The clarification of the difference of fade will help to achieve an appropriate dosage regimen. The magnitude of blockade is modulated by the affinities of drugs and the transmitter acetylcholine (ACh) for the postsynaptic receptors as well as the concentrations of the drugs and ACh. The purpose of this study is to propose an explanation of the relationship between the degree of TOF fade and the potencies of the drugs using a dynamic model based on the law of mass action.

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Theory

Because nondepolarizing neuromuscular blocking drugs compete with ACh for the postsynaptic nicotinic receptors, the concentrations of ACh-receptor complex (RA) and drug-receptor complex (RC) in a neuromuscular junction are written by Eqs. (1) and (2), respectively, applying expressions used in competitive enzyme kinetics:

$$RA = R_T \frac{A}{A + K_A(1 + C/K_C)} \quad (1)$$

$$RC = R_T \frac{C}{C + K_C(1 + A/K_A)} \quad (2)$$

where R_T is the total concentration of the receptor, and A and C are the unbound concentrations of ACh and drug, respectively. K_A and K_C are the dissociation constants for the receptor complex of ACh and the drug. The total concentration of ACh (A_T, i.e., the concentra-

tion of released ACh) and drug (C_T) are written by Eqs. (3) and (4), respectively:

$$A_T = A + RA \tag{3}$$

$$C_T = C + RC. \tag{4}$$

Substituting Eqs. (3) and (4) into Eqs. (1) and (2), R_A and R_C can be written as follows:

$$RA = R_T \frac{A_T - RA}{A_T - RA + K_A\left(1 + \frac{C_T - RC}{K_C}\right)} \tag{5}$$

$$RC = R_T \frac{C_T - RC}{C_T - RC + K_C\left(1 + \frac{A_T - RA}{K_A}\right)} \tag{6}$$

It was assumed that the concentration of ACh released by the fourth stimulus in a train (A_{T4}) is lower than the concentration of ACh released by the first stimulus (A_{T1}), irrespective of the presence or absence of a drug, because ACh mobilization in a motor nerve is time-dependent [12] and the ACh molecules released by each stimulus are immediately metabolized after binding to the receptors and causing a muscle contraction [13].

The receptor-binding of ACh evokes the twitch response of a muscle. In neuromuscular transmission, the margin of safety exists and the maximum twitch response is evoked at submaximal receptor occupancy by ACh [14]. Hill's equation can accommodate such a nonlinear transduction of the receptor occupancy by an agonist into the pharmacological effect [15]. Hence, to describe the relationship between the receptor occupancy and twitch response, the twitch height (TW) is related to RA with Hill's equation as follows:

$$TW = TW_{MAX} \frac{RA^s}{RA^s + RA_{50}{}^s} \tag{7}$$

where TW_{MAX} is the physiological maximum twitch height, RA_{50} is the concentration of the ACh-receptor complex which causes 50% twitch height of TW_{MAX} and s is Hill's coefficient.

Methods

Simulation with the model was carried out on a personal computer (PC-9801/RA, NEC, Tokyo, Japan). The influence of difference of K_C on the T1-TOF ratio relationship as well as the drug concentration-muscle relaxation relationship was studied. The parameter values other than K_C and C_T were fixed to the reported values [6,7] shown in Table 1. For the simulation, RA and RC in Eqs. (5) and (6) were first solved numerically using Newton's iterative method after specific values of K_C and C_T were given. Then twitch heights in the presence or absence of a drug were obtained by using Eq. (7), and the twitch height ratio (the ratio of a twitch height to the control value) and the TOF ratio (the ratio of the fourth twitch height in a train to the first twitch height at a certain drug concentration) were calculated.

Results

Relationships between drug concentration and twitch height ratios of T1 and T4 were simulated using various values of K_C, the results of which are shown in Fig. 1. The T1-T4 relationship and the T1-TOF ratio relationship are also presented in Fig. 2. The TOF ratio in the absence of a drug was estimated to be almost 1.0. Irrespective of the value of K_C, T4 at a certain drug concentration decreased more than T1 as shown in Fig. 1. As the value of K_C increased, the drug concentration-twitch height curves of T1 and T4 shifted to the right (Fig. 1) and the T1-T4 ratio curves became concave (Fig. 2A). The characteristics of T1–T4 curves denoted that an increase of K_C augments the degree of T4 depression at a fixed T1 depression. Consequently, an increase of K_C reduced the TOF ratio as shown in Fig. 2B. It was

Table 1. Parameter values used for simulation

Parameter	Value
K_A (μM)	0.100
A_{T1} (μM)	0.866
A_{T4} (μM)	0.610
R_T (μM)	0.205
RA_{50} (μM)	0.0665
s	8.56

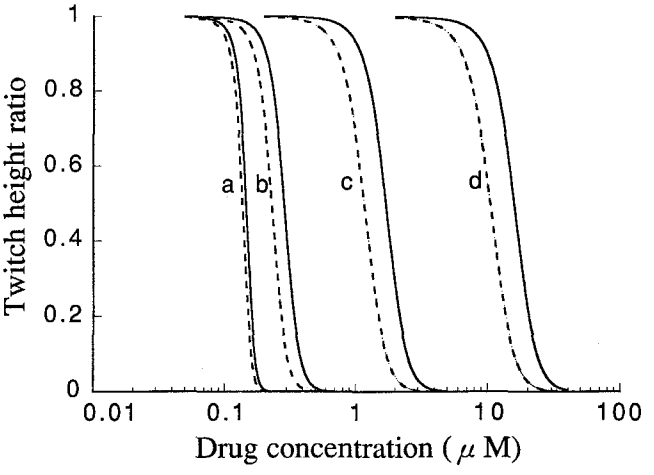

Fig. 1. Drug concentration-twitch height ratio relationship of T1 and T4. The twitch height ratio denotes the ratio of T1 or T4 to the value of each in the absence of drugs. *Solid* and *dashed lines* represent the twitch height ratios of T1 and T4, respectively. Values of K_C used for the simulation are *a*, 0.001; *b*, 0.01; *c*, 0.1; *d*, 1.0 (μM). The abscissa is expressed on logarithmic scale

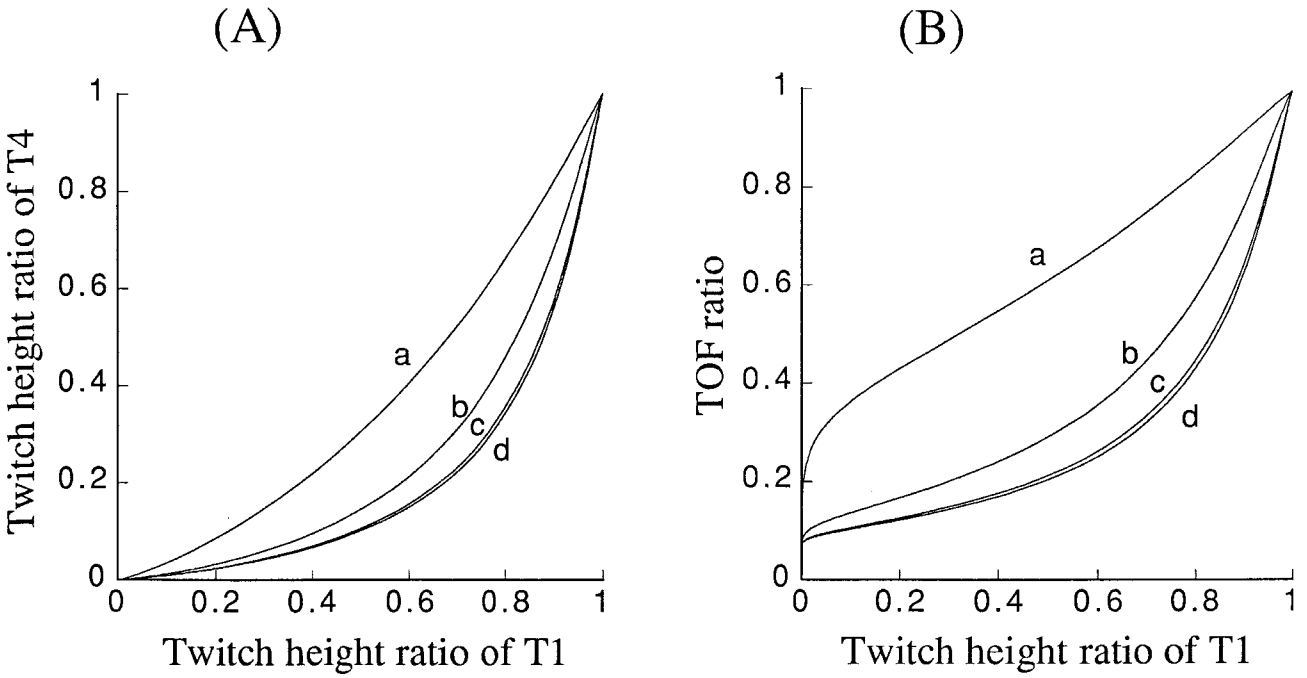

Fig. 2A,B. T1–T4 relationship and T1-TOF ratio relationship. The TOF ratio denotes the ratio of T4 in a train to T1. **A** Relationship between twitch height ratios of T1 and T4. **B** Relationship between twitch height ratio of T1 and TOF ratio. Values of K_C are *a*, 0.001; *b*, 0.01; *c*, 0.1; *d*, 1.0 (μM)

predictable that the degree of fade is inversely correlated with the potencies of drugs.

Discussion

Vecuronium (VEC), pancuronium (PAC), alcuronium (ALC), atracurium (ATR), *d*-tubocurarine (*d*-TC), fazadinium (FAZ), and gallamine (GAL) have been used as nondepolarizing neuromuscular blocking drugs for clinical and research purposes. The order of the in vivo potencies is VEC > PAC > ALC > ATR > *d*-TC > FAZ > GAL [16]. Williams et al. [3] compared the degree of TOF fade in human subjects and reported that the degree of fade was PAC < ALC < *d*-TC < FAZ < GAL. Gibson and Mirakhur [4] demonstrated that the degree of TOF fade in human subjects was VEC = PAC < ATR = *d*-TC. Klein et al. [5] found that the degree of fade in horses was VEC < PAC = GAL. These reported orders of the degree of TOF fade were almost in inverse relation to the order of the potencies of the drugs.

The difference of TOF fade induced by nondepolarizing neuromuscular blocking drugs has been attributed to a presynaptic action of the drugs [8–10]. At the presynaptic level, the drugs inhibit the feedback control of ACh release and reduce the ACh output at the fourth stimulus in a train in comparison with that at the first stimulus. However, it has been reported that *d*-TC fails to inhibit ACh output from a nerve terminal under train stimulations of 15 pulses at 5.0 Hz [11], suggesting that the presynaptic action is not operative under the TOF stimulations at usual frequencies lower than 4.0 Hz. A more feasible explanation of the difference of TOF fade is necessary.

Our previous work suggested that the time-dependent ACh mobilization results in less ACh output at the fourth stimulus than at the first stimulus [6,7]. The nondepolarizing neuromuscular blocking drugs share similar pharmacokinetic properties because of their similar physicochemical characteristics [17]. Thus, it is conceivable that the magnitude of the affinities for the postsynaptic receptors is responsible for the difference of TOF fade.

For a drug with a low affinity, a considerable amount is necessary to compete with ACh for the receptor and to depress twitch responses. It is assumed that the fraction of drug bound to the receptor is negligible with respect to the total concentration of the drug. Thus, C in Eq. (1) tends to be constant and the apparent affinity for the formation of ACh-receptor complex (RA) is hardly affected by the reduction of ACh output through the first to the fourth stimulus in a train. Subsequently, RA directly reflects the change of ACh output. The reduction of ACh output diminishes the receptor occupancy by ACh. In this way, the drug concentration-relaxation curves of T1 and T4 separate from each other and the TOF fade is observed (Figs. 1, 2).

On the other hand, the degree of TOF fade decreases in the presence of a drug with a high affinity. In this

case, the fraction of drug bound to the receptor becomes nonnegligible with respect to the total drug, and then the unbound concentration of the drug is no longer proportional to the total concentration. When ACh output decreases, the resulting increase in receptor occupancy of the drug concurrently leads to a significant decrement of C relative to C_T and augments the apparent affinity for the formation of RA, i.e., the inverse of K_A $(1 + C/K_C)$ in Eq. (1). This augmentation of the affinity counteracts the reduction of ACh output for the maintenance of receptor occupancy by ACh. Eventually, the difference in RA at both stimuli becomes smaller and the twitch heights of T1 and T4 become similar.

The pharmacological potency is generally assumed to be directly proportional to the affinity for the receptors associated with the drug effect. However, in neuromuscular transmission, the relation is not always valid. The potency ratio of PAN/GAL is reported to be 40 [16], whereas the examination of ACh-elicited currents for the mouse muscle ACh receptor [18] showed the affinity ratio of PAN/GAL to be about 400. The present results also account for the relationship between the potency and the affinity for the receptor. In Fig. 1, the EC_{50} of a drug with $K_C = 1.0\,\mu M$ for T1 depression is about $10\,\mu M$, whereas that with $K_C = 0.001\,\mu M$ is $0.1\,\mu M$. Then the 1000-fold difference in the affinity for the receptor would lead to a potency ratio of 100. If in all instances the unbound concentration of a drug adjacent to the receptor site is nearly equal to that of the total drug, these results could not be observed. The twitch response is evoked even at submaximal receptor occupancy by ACh and a drug must occlude a large portion of the receptors to block the muscle contraction. When the receptors densely exist on a motor end-plate, the formation of the drug-receptor complex would be accompanied by a decrease in the amount of the unbound drug available for promoting receptor occlusion. Subsequently, even when a drug has a fairly high affinity, a total drug concentration of at least more than the receptor concentration is necessary to exert the blockade effect. Thus, the discrepancy between the potency and the affinity for the receptors would be much greater for high-affinity drugs.

In previous reports, we showed that the maximum twitch responses at the first and fourth stimuli in the absence of a drug and the faint TOF fade by α-bungarotoxin could be primarily explained in terms of the existence of the margin of safety [6,7]. Nondepolarizing neuromuscular blocking drugs competitively inhibit the ACh-receptor binding and produce the blockade effect. Therefore, in this study, we constructed a dynamic model including the receptor occupancy by ACh, the competitive inhibition of the receptor-ACh binding by drugs, the difference of affinities of the drugs for the receptors and the transduction of ACh-receptor binding to a muscle contraction. As a result, it was demonstrated that the pharmacological response mediated through the densely existing receptors would be modified by the unbound fraction rather than the total drug amount.

To date, in researches on the difference of TOF fade induced by the drugs, the difference of affinities of the drugs for the postsynaptic receptors has not been taken into account. Additionally, although several kinetic studies on the pharmacological effect of non-depolarizing neuromuscular blocking drugs have been documented [19–21], the difference of TOF fade among the drugs has not been clarified. The present study, however, pointed out that TOF fade is not always an indicator of the presynaptic action of a drug. Rather, the affinity of the drug for the postsynaptic receptors may also govern the degree of TOF fade.

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