Kinetic Analysis of Neuromuscular Blockade, II.
Train-of-Four Fade Induced by d-Tubocurarine and α-Bungarotoxin

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The degree of train-of-four (TOF) fade, i.e., the reduction of the fourth to the first twitch height in a train, induced by d-tubocurarine (d-TC) and α-bungarotoxin (α-BX) was investigated. The fade induced by d-TC was pronounced in comparison with that by α-BX, and the difference was analyzed using a kinetic model. Based on the assumptions: (1) Acetylcholine (ACh) binds to the nicotinic receptor and evokes twitch response. (2) The amount of released ACh is dependent on stimulus interval. (3) d-TC interacts competitively with the receptor. (4) α-BX interacts irreversibly with the receptor. It was suggested that the fade by d-TC and α-BX can be explained by the difference of the receptor occupancy by ACh which was caused by different interaction mechanisms of the two muscle relaxants with receptors.

Keywords: train-of-four fade; d-tubocurarine; α-bungarotoxin; acetylcholine; pharmacodynamics; pharmacokinetics

Train-of-four (TOF) stimulation at 2.0 Hz is often used in a clinical setting.1–3 The TOF fade, i.e., the reduction of the fourth to the first twitch height in a train, becomes more pronounced after administration of d-tubocurarine (d-TC) than after α-bungarotoxin (α-BX),4,5 a snake venom, which binds to a nicotinic receptor at a motor end-plate specifically and irreversibly.6–8 The difference of TOF fade induced by d-TC and α-BX has not yet been explained quantitatively.

We earlier reported that twitch height depression under TOF stimulation after d-TC administration can be explained by a pharmacokinetic/pharmacodynamic model based on a hypothesis that the released amount of neuromuscular transmitter, acetylcholine (ACh), is dependent on stimulus interval.9 In the present report, we applied the same hypothesis to the pharmacological effect of α-BX and attempted to evaluate the TOF fade by d-TC and α-BX on the basis of their different interaction mechanisms with receptors.

THEORETICAL

Receptor Occupancy by ACh and α-BX Because α-BX binds a nicotinic acetylcholine receptor irreversibly and acts as a noncompetitive antagonist in the neuromuscular transmission,6–8,10 it was assumed that ACh interacts with the receptor not occupied by α-BX. The concentration of ACh–receptor complex (RA) in an action site is given by Eq. 1:

\[ RA = R_A(1 - X) \frac{A}{A + K_A} \]  

(1)

where \( R_A \) is the total receptor concentration, \( X \) is the ratio of receptors occupied by α-BX to the total receptors, \( A \) is the unbound ACh concentration and \( K_A \) is the dissociation constant for ACh. The total ACh (\( A_T \)), i.e., concentration of ACh released by one electrical stimulus, is written by Eq. 2 as described previously9:

\[ A_T = A + RA \]  

(2)

Substituting Eq. 2 into Eq. 1, \( RA \) can be written as follows;

\[ RA = R_A(1 - X) \frac{A_{T} - RA}{A_{T} - RA + K_A} \]  

(3)

Receptor Occupancy by ACh, d-TC and α-BX It was assumed that d-TC competes with ACh for the receptor not occupied by α-BX. Then, ACh–receptor complex (RA) and d-TC–receptor complex (RC) concentrations are given by Eqs. 4 and 5, respectively:

\[ RA = R_A(1 - X) \frac{A}{A + K_A(1 + C/K_C)} \]  

(4)

\[ RC = R_A(1 - X) \frac{C}{C + K_A(1 + A/K_A)} \]  

(5)

where \( C \) is the unbound d-TC concentration and \( K_C \) is the dissociation constant for d-TC. The total concentrations of ACh (\( A_T \)) and d-TC (\( C_T \)) are given by Eqs. 2 and 6, respectively:

\[ C_T = C + RC \]  

(6)

Substituting Eqs. 2 and 6 into Eqs. 4 and 5, \( RA \) and \( RC \) can be written as follows;

\[ RA = R_A(1 - X) \frac{A_{T} - RA}{A_{T} - RA + K_A(1 + C_T/K_C)} \]  

(7)

\[ RC = R_A(1 - X) \frac{C_{T} - RC}{C_{T} - RC + K_A(1 + A_{T} - RA/K_A)} \]  

(8)

ACh Release and Twitch Height Response As described previously9, it was assumed that the released ACh concentration (\( A_T \)) depended on stimulus interval, i.e., \( 0.610 \times 10^{-6} \text{M} \) at 0.5 s interval, \( 0.866 \times 10^{-6} \text{M} \) at 10.5 s interval. The twitch height (TW) was related to the receptor occupancy by ACh (\( RA \)) with Hill's equation as follows:

\[ TW = TW_{max} \frac{RA}{RA + RA_{50}^{*}} \]  

(9)

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where \( T_{W_{\text{max}}} \) is the physiological maximum twitch height, \( R_{A_{50}} \) is the concentration of the ACh-receptor complex which causes 50% twitch height of \( T_{W_{\text{max}}} \) and \( s \) is Hill's coefficient.

MATERIALS AND METHODS

Materials  
d-Tubocurarine chloride and \( \alpha \)-bungarotoxin were purchased from Yoshitomi Pharmaceutical Co., Japan and Wako Pure Chemicals, Japan, respectively. The other chemicals were of reagent grade.

Twitch Height Measurement  
Male Wistar rats (180—220 g) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Twitch heights were measured according to the method reported previously.\(^{9,11} \) TOF stimulation (frequency of 2.0 Hz) repeated every 12 s was used, and a series of four twitch heights (T1, T2, T3 and T4) was measured continuously before and after drug administration. Unless otherwise stated, supramaximal voltage was used.

Drug Administration  
The twitch heights were measured after administration of \( \alpha \)-BX (0.05, 0.15, 0.25 mg/kg, i.v.) and after coadministration of \( \alpha \)-BX (0.05 mg/kg, i.v.) and \( d \)-TC (0.05 mg/kg, i.v.). In the coadministration experiments, \( d \)-TC was injected 20 or 80 min after \( \alpha \)-BX administration. The coadministration experiments are hereafter referred to as condition I and II, respectively.

The twitch height depression induced by drugs was represented as the ratio (R1 or R4) of the first or fourth twitch heights in a train after drug administration to the corresponding control values. TOF ratio (T4/T1) was used as the index of TOF fade.

Stimulating-Voltage-Dependency of Twitch Heights

The twitch heights evoked at various stimulating voltages (2.5—7.0 V) were measured in the control study and 20 min after administration of \( \alpha \)-BX (0.05 mg/kg, i.v.).

Data Analysis  
Data were analyzed based on the model described in the Theoretical section. The time courses of R1 and R4 in the coadministration experiments were simultaneously fitted to Eqs. 7 and 8 using a nonlinear least squares program MULTII\(^{12} \) and the value of parameter \( X \) was estimated. The calculation method was similar to that described.\(^9 \) The parameters other than \( X \) were fixed to the values of the report.

RESULTS

Twitch Height Responses after \( \alpha \)-BX Administration  
As shown in Fig. 1, the twitch heights are irreversibly depressed by \( \alpha \)-BX with faint fade.

The dose-dependent twitch height depression after \( \alpha \)-BX administration is shown in Fig. 2. The twitch heights were completely depressed after administration of 0.15 and 0.25 mg/kg \( \alpha \)-BX. For 0.05 mg/kg of \( \alpha \)-BX, although the twitch height began to decrease slightly at about 40 min, it remained constant after 60 min. The TOF ratio was not reduced less than 55% over the dose range of 0.05 to 0.25 mg/kg.

Twitch Height Responses after Coadministration  
The twitch heights (R1 and R4) after 0.05 mg/kg \( d \)-TC administration in the presence and absence of \( \alpha \)-BX are shown in Fig. 3. The twitch height depression by \( d \)-TC was more pronounced and elongated in the coadministration with \( \alpha \)-BX. The degree of depression was more intense under condition II than under condition I. Irrespective of the presence or absence of \( \alpha \)-BX, the addition of \( d \)-TC
induced the departure of R1 and R4, i.e., TOF fade. The model-predicted values are also shown in Fig. 3 as solid curves, and are in good agreement with the observed data. The estimated values of X in conditions I and II were $0.247 \pm 0.003$ (mean ± S.D.) and $0.453 \pm 0.004$, respectively.

**Effect of α-BX on Stimulating-Voltage-Dependency of Twitch Heights** The evoked twitch heights increased with an increase in stimulating voltage (Fig. 4). T1 and T4 in the presence of α-BX were both significantly lower than the corresponding control values at low voltage (2.5—4.0 V). At the supramaximal voltage larger than 4.5 V, however, there was little difference between the twitch heights in the presence and absence of α-BX.

**DISCUSSION**

The faint fade by α-BX in the present study is in striking contrast to the pronounced fade by d-TC in the previous report. A similar contrast of TOF fade between the two relaxants has been observed. The difference of fade implied that ACh released from a motor nerve was not reduced in the presence of α-BX but was in the presence of d-TC. Subsequently, this difference has been regarded
as a phenomenon supporting the view that d-TC acts on the prejunctional nicotinic acetylcholine receptors and reduces the ACh output from the nerve.\textsuperscript{4,5} Wessler \textit{et al.}, however, observed that d-TC failed to inhibit the ACh release evoked by trains of 15 pulses at 5 Hz.\textsuperscript{13} This observation argues against the opinion that the fade is mediated exclusively by the presynaptic action of d-TC.

To understand the difference of the TOF fade by d-TC and \(\alpha\)-BX, we thought it necessary to consider the receptor occupancy by ACh in the presence of the relaxants as well as the released ACh amount, because the twitch tension results from the receptor-binding of ACh. For ACh output by electrical stimulations, it was assumed that the reduction of released ACh amount occurs in a train under TOF stimulation; however, this event is not a consequence of the action of d-TC but is, rather, manipulated by the time-dependent ACh mobilization.\textsuperscript{9}

The assumption regarding the receptor-binding of antagonists was the simplest one and generally accepted: d-TC competes with ACh for the receptors, whereas \(\alpha\)-BX binds to them irreversibly and inhibits the binding of ACh noncompetitively.

The transduction of the receptor-binding of ACh into the pharmacological effect, however, was more complicated. As Fig. 4 shows, the twitch response is dependent on the stimulating voltages. A similar relationship has also been reported in human subjects.\textsuperscript{14} Because the released ACh increases with electrical stimulus intensity,\textsuperscript{15,16} the stimulating voltage can be used as an index of the amount of ACh released. \(\alpha\)-BX elicited the twitch height depression at a low voltage. At the supramaximal voltage evoking maximal twitch heights, however, \(\alpha\)-BX could produce no additional effect. It was apparent that \(\alpha\)-BX still occupied a part of the receptors at the supramaximal voltage, because d-TC induced a more intense blockade in the coexistence with \(\alpha\)-BX (Fig. 3). This result suggested the existence of the margin of safety.\textsuperscript{17–19} Therefore, Eq. 9 was used to describe the transduction of the receptor-occupancy of ACh into the pharmacological effect (Eq. 9).

Using the model, an attempt was made to fit the time courses of twitch height depression in the coexistence of the two relaxants. As Fig. 2 shows, the twitch heights (R1 and R4) up to 40 min after administration of 0.05 mg/kg \(\alpha\)-BX were almost the same as the control values. After the following gradual depression, the height became a constant level again at about 60 min; 0.05 mg/kg of d-TC, therefore, was injected during each distinct period with the constant twitch height corresponding to condition I or II. The model-predicted values are superimposed on the observed data in Fig. 3. The twitch height depression by d-TC could also be explained by the constant receptor occupancy by \(\alpha\)-BX under each condition: \(X\) was 0.247 and 0.453 for d-TC single administration, conditions I and II.

The expected relationship between twitch height depression and receptor occupancy by \(\alpha\)-BX is illustrated in Fig. 5, where the twitch heights and the TOF ratio at \(X=0.247\) do not differ from the respective control values. At \(X=0.453\), although both R1 and R4 are slightly reduced, the TOF ratio is barely altered. The figure also indicates that even if the twitch heights are completely depressed by \(\alpha\)-BX, the TOF ratio never drops below 0.65. The illustration shows a striking resemblance to the observed data for 0.05, 0.15 and 0.25 mg/kg \(\alpha\)-BX (Fig. 2).

Figure 6 depicts how the change of released ACh concentration influences twitch depression by \(\alpha\)-BX or d-TC. Because the dissociation constant of ACh for the receptor (\(K_D\)) is \(0.1 \times 10^{-6}\) M and pharmacological shift ratio (\(K_D/R_A_{50} = 3.1\)) is not large,\textsuperscript{9} the saturation in receptor-binding of ACh and the nonlinear transduction of the receptor occupancy into the effect are both involved in these twitch height responses. In the absence of

![Graph](image-url)
antagonist, as the released ACh concentration increases, twitch tension increases and reaches the maximum intensity. The $a$-BX and $d$-TC depress twitch heights by inhibiting the receptor-binding of ACh noncompetitively and competitively, respectively. When part of the receptors is occluded by $a$-BX, if the released ACh is less than about twice $K_A$, twitch height is lower than the corresponding control value. When the released ACh exceeds $K_A$, however, maximum twitch response is evoked as in the control experiment as long as the receptor occupancy by $a$-BX is within about 50%. The situation in excess ACh release also gives us a feasible explanation of the faint TOF fade by $a$-BX. The affinity of ACh for the receptors is not altered in the presence of $a$-BX. Although the time-dependent mobilization causes smaller ACh output at the fourth stimulus in a train than at the first stimulus, the ACh concentrations released at both stimuli at supramaximal voltage are large enough to almost saturate the receptors not occupied by $a$-BX. The margin of safety and the saturation of receptor occupancy by ACh cause similar twitch heights at T1 and T4, that is, the faint TOF fade by $a$-BX. Thus, in the presence of $a$-BX, when ACh output exceeds $K_A$, twitch heights hardly reflect the change of ACh concentration but are exclusively governed by the receptor occupancy by $a$-BX. On the other hand, $d$-TC alters the affinity of ACh for the receptor. For the reason, the change of concentration of ACh as well as $d$-TC is a factor governing the degree of the twitch depression by $d$-TC, even when the ACh concentration exceeds $K_A$. As a result, striking fade is produced by $d$-TC under the time-dependent mobilization of ACh.

Consequently, it is apparent in Fig. 6 that twitch depression is less sensitive to the change of ACh output in the presence of a noncompetitive relaxant than a competitive one. In accordance with the model, a similar contrast of TOF fade would be expected between the other competitive and noncompetitive relaxants. Actually, striking TOF fade has been observed by competitive drugs such as pancuronium, atracurium and vecuronium. On the other hand, erabutoxin b which binds to the receptor irreversibly like $a$-BX produces no fade during the twitch depression. No kinetic analyses have yet been done on the contrast of the TOF fade and the interaction mechanisms of relaxants with receptors have not been given attention. This study, however, demonstrated that the absence of TOF fade by noncompetitive relaxants is not always evidence of constant ACh output. Furthermore, the difference of the interaction mechanisms with receptors has turned out to be a possible factor causing the difference of TOF fade under the time-dependent ACh mobilization. Finally, the present study supported the suggestion of Wessler et al., that the TOF fade by $d$-TC is not a consequence of the presynaptic action. The margin of safety as well as the noncompetitive antagonism of $a$-BX might have masked the time-dependent ACh mobilization and resulted in little change of TOF ratio in the absence or presence of $a$-BX.

In conclusion, the kinetic analysis manifested that the interaction mechanisms of relaxants with receptors should be considered in the study of TOF fade. The difference of TOF fade induced by $d$-TC and $a$-BX was attributed to their different interaction mechanisms with receptors. The ACh output under TOF stimulation at 2.0 Hz must be reduced through the time-dependent ACh mobilization rather than the presynaptic action of $d$-TC.

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