

Different Modes of Potentiation by β -Eudesmol, a Main Compound from *Atractylodes Lancea*, Depending on Neuromuscular Blocking Actions of *p*-Phenylene-Polymethylene Bis-Ammonium Derivatives in Isolated Phrenic Nerve-Diaphragm Muscles of Normal and Alloxan-Diabetic Mice

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Received February 21, 1992 Accepted June 8, 1992

ABSTRACT—The essential moieties in *p*-phenylene-polymethylene bis-ammonium (PMBA) derivatives, $C_6H_4[X(CH_2)_nN^+R_3]_2$, on the potentiating effects by β -eudesmol, a main component of *Atractylodes lancea*, of their neuromuscular blockades were investigated in isolated phrenic nerve-diaphragm muscle preparations of normal and alloxan-diabetic mice. PMBA derivatives were separated into the following three groups based on the patterns of the potentiating effects: group I: PMBA-23 ($n = 6$, $R = Me$) and PMBA-24 ($n = 6$, $R = Et$); group II: PMBA-1 ($n = 4$, $R = Me$), PMBA-21 ($n = 4$, $R = Et$) and PMBA-2 ($X = O$, $n = 3$, $R = Me$); and group III: PMBA-31 ($X = S$, $n = 3$, $R = Me$), PMBA-3 ($X = CO$, $n = 3$, $R = Me$) and PMBA-4 ($X = CHOH$, $n = 3$, $R = Me$). The pretreatment with $80 \mu M$ β -eudesmol for 60 min did not affect group I-induced neuromuscular blocking action, and it potentiated group II- and group III-induced ones. The potentiating effect of β -eudesmol on group III was greater in diabetic muscles than in normal ones and that on group II was to the same extent in both muscles. These results suggest that the four-methylene length of the side chains in normal muscles and the hydrophilic moieties adjacent to a phenylene ring in diabetic muscles are related to the potentiating effect by β -eudesmol on PMBA derivatives.

Keywords: β -Eudesmol, *p*-Phenylene-polymethylene bis-ammonium, Diabetic mice, Neuromuscular blockade

The change of sensitivity to various neuromuscular blockers in the neuromuscular transmission of diabetic animals has been demonstrated. Succinylcholine (SuCh), a depolarizing blocker, inhibits the neuromuscular transmission with a higher sensitivity in diabetic skeletal muscles than in normal ones (1–3). Another depolarizing blocker, decamethonium (C_{10}), inhibits it with the same sensitivity in both muscles (2, 4) or rather with a lower sensitivity in diabetic motor endplates (3). We have previously reported that β -eudesmol, a compound from *Atractylodes lancea rhizoma*, potentiates the neuromuscular blocking effects of

SuCh and C_{10} but not that of the competitive blocker pancuronium and that the potentiating effects are greater in diabetic muscles than in normal ones (2). Single channel recordings indicate that β -eudesmol blocks the nicotinic acetylcholine-receptor channels in both open and closed conformations in normal skeletal muscle cells (5). To clarify the essential chemical structure in SuCh blockade potentiated by β -eudesmol in diabetic muscles, we synthesized unhydrolyzable compounds, *p*-phenylene-polymethylene bis-ammonium (PMBA) derivatives. Thus, the present aim is to study the structure-activity relationship of PMBA derivatives producing the potentiating effect by β -eudesmol in normal and diabetic skeletal muscles.

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MATERIALS AND METHODS

Male mice (ddY strain, 4 weeks) were injected with alloxan monohydrate (85 mg/kg, Nacalai Tesque) into the tail vein and were used at 4–5 weeks after the injection. The phrenic nerve-diaphragm muscles of normal (28–36 g) and alloxan-diabetic (24–39 g, feeding blood glucose level: 360–600 mg/dl) mice were isolated. The isometric twitch tensions were obtained under 1 g loading tension, by stimulating the phrenic nerve and the muscle, alternately (0.2 Hz, 1 msec duration, supramaximal voltage), through a bipolar platinum electrode. Each PMBA derivative was applied cumulatively at 2-min intervals after pretreatment with β -eudesmol for 60 min. The inhibitory responses were represented as a percentage of the control value for 1 min before applying the PMBA derivative. β -Eudesmol were dissolved in propylene glycol at the final concentration of 0.2% (V/V). This concentration of propylene glycol did not affect the twitch response. The control response of each PMBA derivative without β -eudesmol was obtained after pretreatment with 0.2% (V/V) propylene glycol.

PMBA derivatives used were as follows: 1,4-bis (4-trimethylammonioethyl) benzene diiodide (PMBA-1), 1,4-bis (3-trimethylammoniopropyloxy) benzene diiodide (PMBA-2), 1,4-bis (4-trimethylammonioethyl) benzene diiodide (PMBA-3), 1,4-bis (1-hydroxy-4-trimethylammonioethyl) benzene diiodide (PMBA-4), 1,4-bis (4-triethylammonioethyl) benzene diiodide (PMBA-21), 1,4-bis (6-trimethylammoniohexyl) benzene diiodide (PMBA-23), 1,4-bis (6-triethylammoniohexyl) benzene diiodide (PMBA-24), 1,4-bis (3-trimethylammoniopropylthio) benzene diiodide (PMBA-31). The synthesis of these PMBA derivatives will be described elsewhere.

The statistical significance was analyzed by Student's *t*-test for unpaired observations at *P* = 0.05 or 0.01.

RESULTS

All PMBA compounds used in this study blocked the nerve-evoked twitch response, without affecting the contraction evoked by stimulation of the muscle. The blocking effects were readily reversed on washout. They were not reversed by 15 μ M neostigmine, a cholinesterase inhibitor, which fully reversed *d*-tubocurarine (6.5 μ M)-induced complete inhibition of indirectly stimulated twitch tension (data not shown).

Blocking effects of PMBA-1 and PMBA-23, with or without β -eudesmol

PMBA-1, a bis-trimethylammonium (BTMA) derivative with *n* = 4, and PMBA-23, a BTMA derivative

with *n* = 6, inhibited the nerve-evoked twitch tension in a concentration-dependent manner (Fig. 1, A and B). The blocking effects of PMBA-1 and PMBA-23 were significantly more potent in diabetic muscles than in normal ones. Pretreatment with 80 μ M β -eudesmol for 60 min potentiated the blocking effect of PMBA-1 to 1.9-fold in normal muscles and to 1.7-fold in diabetic ones. This concentration of β -eudesmol by itself did not affect twitch tensions. Pretreatment with β -eudesmol did not affect the blocking effect of PMBA-23 (Table 1).

Blocking effects of PMBA-21 and PMBA-24, with or without β -eudesmol

In both normal and diabetic skeletal muscles, the neuromuscular junction was similarly blocked by PMBA-21, a bis-triethylammonium (BTEA) derivative with *n* = 4, and PMBA-24 (a BTEA derivative with *n* = 6), with no apparent differences in effect (Fig. 2, A and B). Pretreatment with β -eudesmol potentiated the blocking effect of PMBA-21, but not that of PMBA-24, to 1.7-fold in both muscles (Table 1).

Blocking effects of PMBA-2, PMBA-31, PMBA-3 and PMBA-4, and its potentiation by β -eudesmol

The blocking effects of PMBA-2 (a BTMA derivative with X = O), PMBA-31 (X = S), PMBA-3 (X = CO) and PMBA-4 (X = CHOH) (Fig. 3: A, B, C and D, respectively) were potentiated by β -eudesmol, to 2.6-, 2.8-, 2.0- and 5.6-fold in normal muscles and to 2.6-, 6.4-, 7.5- and 8.5-fold in diabetic ones, respectively. The extents of potentiation for PMBA-2 were not dif-

Table 1. Potentiating effect of β -eudesmol on the neuromuscular blocking action of *p*-phenylene-polymethylene bis-ammonium compounds, p -C₆H₄[(CH₂)_nN⁺R₃]₂, in normal and alloxan-diabetic mice

	Compounds		Alone N/D ^{a)}	Potentiation by β -eudesmol		
	n	R		N/D ^{a)}	N ^{b)}	D ^{b)}
PMBA-1	4	Me	1.5**	1.3**	1.9##	1.7##
-23	6	Me	1.4**	1.2	1.0	0.86
-21	4	Et	0.95	0.98	1.7##	1.7##
-24	6	Et	1.0	1.2	0.99	1.2
Decamethonium ^{c)}			0.88	1.8**	3.9##	8.0##
Succinylcholine ^{c)}			1.3**	2.9**	2.7##	6.0##
Pancuronium ^{c)}			1.0	1.0	1.1	1.1

^{a)}IC₅₀ ratio of normal (N) to diabetic (D) mice. ^{b)}IC₅₀ ratio of absence to presence of 80 μ M β -eudesmol. ^{c)}Data were taken from ref. (2). **: *P* < 0.01, significantly more potent in diabetic mice than in normal mice. ##: *P* < 0.01, significantly potentiated by β -eudesmol.

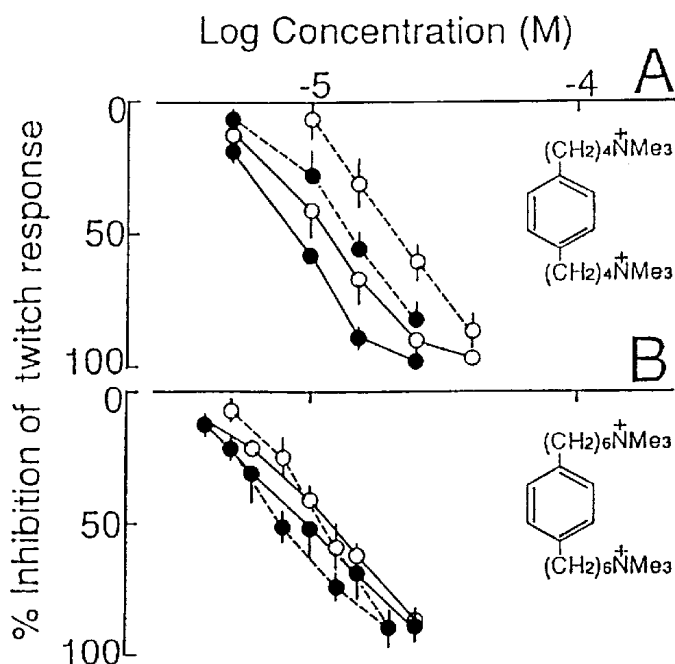


Fig. 1. The potentiating effect of β -eudesmol on the neuromuscular blocking actions of PMBA-1 (A) and PMBA-23 (B) in phrenic nerve-diaphragm muscles of mice. The percent inhibitions of the twitch response in normal (open circles) and alloxan-diabetic (closed circles) mice pretreated with (solid lines) or without (broken lines) $80 \mu\text{M}$ β -eudesmol for 1 hr are plotted against the log concentration of PMBA derivatives. IC_{50} (95% confidence limits, μM): A: 21.1 (17.5–25.3, --○--), 11.0 (9.21–13.1, —○—), 14.1 (11.8–16.9, --●--), 8.37 (7.84–8.94, —●—); B: 11.5 (9.93–13.2, --○--), 11.3 (10.4–12.4, —○—), 8.42 (7.30–9.71, --●--), 9.82 (8.04–12.0, —●—). Values are means \pm S.E.M. ($n = 4-6$).

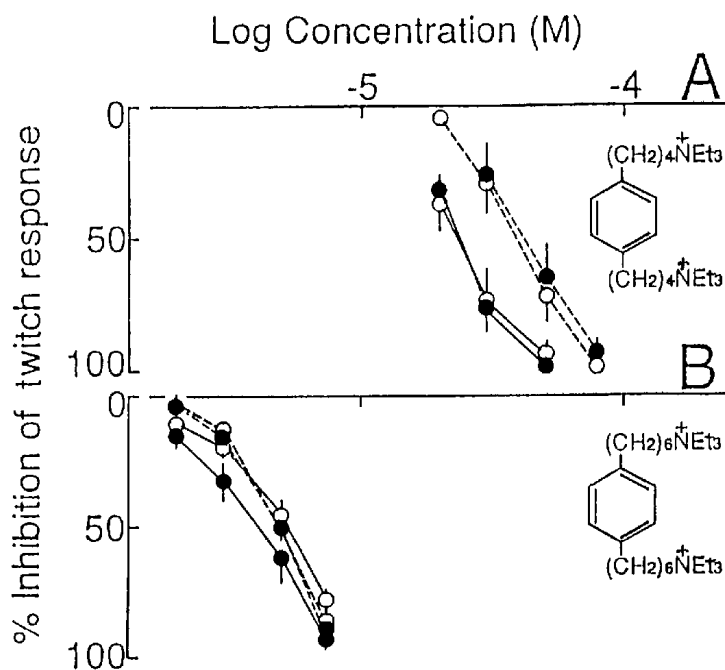


Fig. 2. The potentiating effect of β -eudesmol on the neuromuscular blocking actions of PMBA-21 (A) and PMBA-24 (B) in phrenic-nerve diaphragm muscles of mice. The percent inhibitions of the twitch response in normal (open circles) and alloxan-diabetic (closed circles) mice pretreated with (solid lines) or without (broken lines) $80 \mu\text{M}$ β -eudesmol for 1 hr are plotted against the log concentration of PMBA derivatives. IC_{50} (95% confidence limit, μM): A: 38.7 (31.9–47.0, --○--), 23.2 (18.7–28.8, —○—), 40.9 (31.2–53.7, --●--), 23.6 (21.3–26.2, —●—); B: 4.85 (4.51–5.22, --○--), 4.89 (4.43–5.40, —○—), 4.74 (4.47–5.04, --●--), 4.05 (3.22–5.08, —●—). Values are mean \pm S.E.M. ($n = 4-6$).

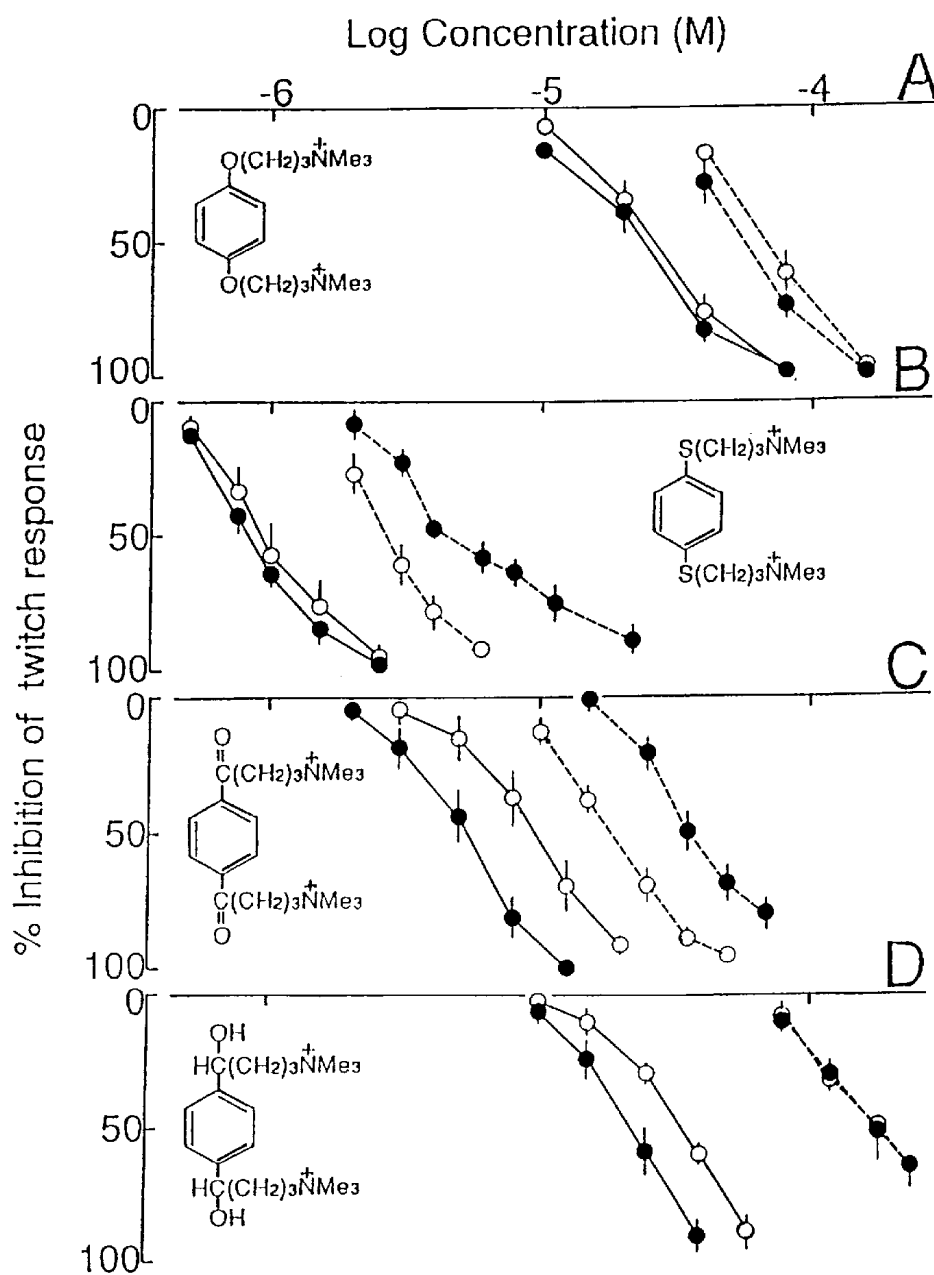


Fig. 3. The potentiating effect of β -eudesmol on the neuromuscular blocking actions of PMBA-2 (A), PMBA-31 (B), PMBA-3 (C) and PMBA-4 (D) in phrenic-nerve diaphragm muscles of mice. The percent inhibitions of the twitch response in normal (open circles) and alloxan-diabetic (closed circles) mice pretreated with (solid lines) or without (broken lines) 80 μ M β -eudesmol for 1 hr are plotted against the log concentration of PMBA derivatives. IC_{50} (95% confidence limit, μ M): A: 66.6 (56.9–77.9, --○--), 26.0 (21.5–31.5, —○—), 55.6 (47.0–65.7, --●--), 21.8 (18.8–25.1, —●—); B: 2.69 (2.40–3.02, --○--), 0.953 (0.764–1.19, —○—), 5.41 (4.62–6.33, --●--), 0.848 (0.793–0.907, —●—); C: 18.5 (17.1–19.9, --○--), 9.48 (7.64–11.8, —○—), 38.3 (33.9–43.3, --●--), 5.11 (4.34–6.00, —●—); D: 183 (166–201, --○--), 32.7 (30.0–35.8, —○—), 188 (148–239, --●--), 22.2 (19.1–25.7, —●—). Values are means \pm S.E.M. ($n = 5-6$).

ferent between normal and diabetic muscles. The extents of potentiation for PMBA-31, PMBA-3 and PMBA-4 were greater in diabetic muscles than in nor-

mal ones, although PMBA-31 or PMBA-3 by itself exhibited weaker effects in diabetic muscles than in normal ones (Table 2).

Table 2. Potentiating effect of β -eudesmol on the neuromuscular blocking action of *p*-phenylene-polymethylene bis-ammonium compounds, p -C₆H₄[X(CH₂)₃N⁺Me₃]₂, in normal and alloxan-diabetic mice

Compounds		Alone	Potentiation by β -eudesmol		
X		N/D ^{a)}	N/D ^{a)}	N ^{b)}	D ^{b)}
PMBA-2	O	1.2	1.2	2.6 [#]	2.6 [#]
-31	S	0.50	1.1	2.8 [#]	6.4 [#]
-3	CO	0.48	1.9 ^{**}	2.0 [#]	7.5 [#]
-4	CHOH	0.97	1.5 ^{**}	5.6 [#]	8.5 [#]
-1	CH ₂	1.5 ^{**}	1.3 ^{**}	1.9 [#]	1.7 [#]

^{a)}IC₅₀ ratio of normal (N) to diabetic (D) mice. ^{b)}IC₅₀ ratio of absence to presence of 80 μ M β -eudesmol. **: P < 0.01, significantly more potent in diabetic mice than in normal mice. #: P < 0.01, significantly potentiated by β -eudesmol.

DISCUSSION

SuCh has been previously shown to inhibit neuromuscular transmission with a higher sensitivity in diabetic state (1–3), and to further inhibit it to a greater extent in the presence of β -eudesmol (2). To clarify the mechanism of hypersensitivity to SuCh potentiated by β -eudesmol in the diabetic state, we synthesized PMBA compounds instead of SuCh, which is promptly hydrolyzed by pseudocholinesterase in vivo. The present study demonstrated that unhydrolyzable PMBA derivatives had various sensitivities to neuromuscular blockade in diabetic skeletal muscles. Therefore, the modification of neuromuscular transmission may be independent of the change of cholinesterase activity in the diabetic state (4, 6). We think rather that it results from an alteration in the post-junctional acetylcholine receptors, because PMBA derivatives-induced neuromuscular blockades in the diabetic state were greatly affected by β -eudesmol, which is a nicotinic acetylcholine receptor channel blocker (5).

BTMA derivatives, but not BTEA derivatives, depolarize the endplate membrane of mouse diaphragm muscles (7), like various series of alkyltrialkylammonium compounds (8–10). However, the potentiating pattern by β -eudesmol was not different between PMBA-1 and PMBA-21 and between PMBA-23 and PMBA-24. These results suggest that PMBA-induced membrane depolarizations are not necessarily related to the potentiating effect by β -eudesmol, but the proper distance between two nitrogen atoms in the bis-ammonium groups of PMBA derivatives is important for producing the potentiating effect by β -eudesmol.

The potentiating patterns by β -eudesmol on the neuromuscular blocking action of the PMBA deriva-

tives in normal and alloxan-diabetic skeletal muscles can be used to classify these compounds into 3 groups: In group I containing PMBA-23 and -24, the blocking effects were not affected by β -eudesmol; In group II containing PMBA-1, -21 and -2, the effects were potentiated by β -eudesmol to the same extent in normal and diabetic muscles; and in group III containing PMBA-31, -3 and -4, the effects were markedly potentiated, and the extent was greater in diabetic muscles than in normal ones. According to our previous study on the potentiating effects by β -eudesmol (2), pancuronium, a competitive neuromuscular blocker, belongs to group I, and SuCh and C₁₀, depolarizing blockers, belong to group III (Table 1). On the other hand, the PMBA derivatives used in this study were not competitive blockers because their blocking effects were not reversed by neostigmine. This classification into 3 groups of PMBA derivatives indicated structural features of PMBA derivatives reflecting the susceptibility to the potentiating effect of β -eudesmol. The compounds belonging to group I have longer methylene chains, and the compounds belonging to group III have hydrophilic moieties adjacent to the phenylene ring. These results suggest that the distance, comparable to a ten-methylene length, between the two nitrogen atoms in the bis-ammonium groups of PMBA derivatives SuCh and C₁₀ is prerequisite for the potentiating effect by β -eudesmol and that the hydrophilic moieties adjacent to the phenylene ring in PMBA derivatives are important for augmenting the potentiating effect by β -eudesmol in diabetic muscles.

In conclusion, this study on the structure-activity relationship of PMBA derivatives on their neuromuscular blockades suggests that the four-methylene length of the side chains in normal muscles and the hydrophilic moieties adjacent to the phenylene ring in diabetic muscles are related to the potentiating effect of β -eudesmol on PMBA derivatives.

Acknowledgments

We are grateful to Dr. K. Naito and Dr. O. Sakuma (Ome Research Laboratories, Tobishi Pharmaceutical, Japan) for the synthesis of PMBA compounds. This work was supported in part by Special Coordination Funds for Promoting Science and Technology from the Science and Technology Agency, Japan (1991).

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