Depolarizing Neuromuscular Blocking Action of Coryneine Derived from Aconite Root in Isolated Mouse Phrenic Nerve-Diaphragm Muscles

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The mode of the neuromuscular blocking action of coryneine (a quaternary ammonium derivative of dopamine) derived from aconite root was investigated in isolated phrenic nerve-diaphragm muscles and denervated diaphragm muscles of mice. Coryneine (20—150 μM) blocked the nerve-evoked twitch response without affecting the contraction evoked by electrical stimulation of the muscle. The blocking effect was reversed by neostigmine, a cholinesterase inhibitor. The electrical charge-response curve on depolarization produced by iontophoretically applied acetylcholine (ACH) at the endplate regions in normal muscles was shifted to the right on decreasing the maximal response by 40 μM coryneine. The double-reciprocal plot revealed that coryneine reduced the apparent affinity of ACh for its receptor on decreasing the maximal response. Coryneine (20 μM—2 mM) itself depolarized the endplate membrane and this effect was reversibly suppressed by 1 and 5 μM pancuronium. Coryneine (30 μM—10 mM) produced contractions of denervated muscles in a concentration-dependent manner and the effects were reduced by 70 mM pancuronium. These results indicate that coryneine is a depolarizing agent and a mixed-type competitive and noncompetitive neuromuscular blocker.

Key words  coryneine; depolarizing neuromuscular block; phrenic nerve-diaphragm muscle

Coryneine is a compound derived from aconite root. 1,2) Aconite is frequently prescribed in Kampo-Hozai (traditional Sino-Japanese pharmacy) in forms such as Keishika-zyu-tu which is used to relieve muscle pain. 3) Coryneine is a quaternary ammonium derivative of dopamine (Fig. 1). The pharmacological actions of coryneine have been mainly reported to involve the cardiovascular system.1,4,5) In the peripheral nervous system, coryneine exhibits a depolarizing neuromuscular blocking action. 4)

The precise mechanism of action of depolarizing neuromuscular blocking agents has not yet been established. Burns and Paton, 5) and Zaimis 7) have demonstrated that these agents interrupt neuromuscular transmission by persistently depolarizing the endplate region. On the other hand, Thesleff, 5,9) Katz and Thesleff 10) have reported that the neuromuscular blockade caused by acetylcholine (ACH), succinylcholine and decamethonium is not due to persistent depolarization, but to desensitization of the nicotinic ACh receptor. They also pointed that the mode of the depolarizing action of succinylcholine and decamethonium were similar to that of ACh. However, Lorkovic and Rüdel 11) reported that chronic denervation did not cause hypersensitivity of succinylcholine-induced depolarization although ACh exhibited such hypersensitivity, suggesting that the depolarizing action of succinylcholine was different from that of ACh.

In the present study, we investigated electrophysiologically the mode of the neuromuscular blocking action of coryneine in isolated mouse phrenic nerve-diaphragm muscles and denervated diaphragm muscles.

MATERIALS AND METHODS

Male normal mice (ddY strain, 30—41 g) and denervated mice (26—38 g) were used. The denervation procedure was as follows: A left unilateral phrenicotomy was performed by removing 1—1.5 cm of the phrenic nerve at the plexus cervicalis of mice under urethane anesthesia. After 14—18 days, the denervated muscle was isolated.

Twitch Tension The isolated phrenic nerve-diaphragm muscle preparations were suspended in 5 ml Krebs-Henseleit solution (KHS: 118 mM NaCl, 5.4 mM KCl, 2.5 mM CaCl2, 0.57 mM MgSO4, 1.2 mM NaH2PO4, 11.1 mM glucose and 12—15.5 mM NaHCO3) bubbled with 95% O2 + 5% CO2 at 36—37°C. The nerve and muscle were stimulated alternately at a rate of 0.2 Hz through a pair of platinum electrodes (1 ms duration, supramaximal voltage). The muscle tension was recorded isometrically under 1 g loading tension as previously reported. 12)

Electrophysiological Techniques A conventional microelectrode technique was used. Modified KHS (137 mM NaCl, 5.0 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 15 mM NaHCO3 and 10 mM glucose) bubbled with 95% O2 + 5% CO2 was continuously perfused at a temperature of 35—37°C. Glass microelectrodes (5—25 MΩ) filled with 3 M KCl were used to measure the resting membrane potential of muscle cells. ACh potentials were recorded at the endplate regions by iontophoretic application of ACh. An endplate with a rapid rising time (<1 ms) for miniature endplate potentials was used for recording. ACh was applied every 5 s (0.2 Hz) by rectangular current pulses (5-50 nA, 10 ms duration) which passed through a microelectrode (100—200 MΩ) filled with 2 M ACh. In

\[ \text{coryneine} \]

Fig. 1. The Chemical Structure of Coryneine

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order to avoid leakage of ACh, a few nA of braking current was applied. The ACh potential amplitude was corrected to a membrane potential of $-80 \text{mV}$ using a reversal potential of $0 \text{mV}$. The changes in membrane potential caused by drugs were measured using a superfusion technique. The muscle strips were suspended in a 5 ml-chamber filled with continuously flowing nutrient solution (1.5 – 3 ml/min) at 35 – 37 °C. The bath solution could be completely changed in a few seconds by means of a six-way tap. In the above nutrient solution, Ca$^{2+}$ was replaced by Mg$^{2+}$ to avoid contraction and a concentration of 0.3 – 1 μM tetrodotoxin was maintained throughout the experiment to block the voltage-dependent Na channel. Only those cells having a resting membrane potential below than $-60 \text{mV}$ were used for recording.

**Contraction of Denervated Muscle** The denervated diaphragm muscles were isolated and suspended in 5 ml modified KHS. The tension of the muscle was recorded isometrically. When the bath solution was replaced with different concentrations of drug solution, the maximal tension was obtained within a few seconds. The cory neine-induced contractions were estimated as a percentage of the contraction for ACh (1.65 mm).

**Drugs** ACh chloride (Dai-ichi, Tokyo), succinylcholine chloride, d-tubocurarine chloride, dopamine hydrochloride (Nacalai tesque, Kyoto), neostigmine methylsulfate, carbamylcholine chloride (Sigma, St Louis, MO, U.S.A.), pancuronium bromide (a gift from Sankyo, Tokyo) and tetrodotoxin (Sankyo, Tokyo) were used. Coryneine chloride ([2-(3,4-dihydroxyphenyl)ethyl]trimethylammonium chloride) was synthesized by Prof. Koizumi and Dr. Uwano (Toyama Medical and Pharmaceutical University, Toyama).

**RESULTS**

**Neuromuscular Blocking Effect of Coryneine** In isolated mouse phrenic nerve-diaphragm muscle preparations, 100 μM coryneine blocked the nerve-evoked twitch response without affecting the contraction which was evoked by stimulation of the muscle (Fig. 2). The blocking effect was reversed by 15 μM neostigmine as in the case of competitive blocking agents (6.5 μM d-tubocurarine and 2 μM pancuronium). The blocking effects of depolarizing agents (50 μM succinylcholine and 100 μM carbamylcholine) were not reversed. The 50% inhibitory concentration of coryneine at 1h after application was found to be 22.4 μM (18.7 – 27.7 μM; 95% confidence limits). Dopamine did not exhibit neuromuscular blockade even at high concentrations (1 – 4 mM; data not shown).

**Inhibitory Effect of Coryneine on ACh-Induced Depolarization** Ten to thirty minutes after introducing 40 μM coryneine into the bath, the values of the resting membrane potential which were recorded at the endplate membrane of normal muscle cells changed from $-67.6 \pm 2.0 \text{mV}$ to $-58.0 \pm 3.8 \text{mV}$ (mean ± S.E.M.; n = 6 endplates/5 muscles).

Coryneine (40 μM) reduced the amplitude of the ACh potential produced by iontophoretical application to the endplate membrane. The values of the corrected ACh potential amplitude in the presence or absence of coryneine were plotted against the log electrical charge for iontophoresis (Fig. 3A). In the presence of coryneine, the electrical charge–response curve shifted to the right on decreasing the maximal response. In the double-reciprocal plot, straight lines were obtained by assuming a Hill coefficient ($n_H$) of 2 (Fig. 3B). The values of the estimated maximal depolarization and 50% effective electrical charge (EEC$_{50}$) of ACh pulse (coulomb; C) in the presence and absence of coryneine were 16.7 mV (14.0 – 20.7 mV; 95% confidence limits), 0.183 nC (0.147 – 0.277 nC) and 21.7 mV (18.1 – 27.0 mV), 0.102 nC (0.0851 – 0.135 nC), respectively. Coryneine increased the EEC$_{50}$ value of the ACh pulse on decreasing the maximal response. Dopamine (1 – 3 mM) had no influence on ACh-induced depolarization (data not shown).

![Fig. 2. A Typical Recording of the Neuromuscular Blocking Effect of Coryneine (CRN, 100 μM) and Its Reversal by Neostigmine (Neo, 15 μM) on Directly- and Indirectly-Elicited Isometric Twitch Responses in Isolated Mouse Phrenic Nerve-Diaphragm Muscle Preparations](image-url)

Phrenic nerve and muscle were stimulated alternately at a rate of 0.2 Hz. The responses by d-tubocurarine (d-TC, 6.5 μM), pancuronium (Panc, 2 μM), succinylcholine (SuCh, 50 μM) and carbamylcholine (Carb, 100 μM) are shown as controls. The application of above drugs blocked the nerve-evoked twitch responses.
Depolarizing Effect of Coryneine in Normal and Denervated Muscles Coryneine depolarized the endplate region in a concentration (20 μM—2 mM)-dependent manner in normal muscle (Fig. 4A and Fig. 5A). The depolarization produced by 0.2 mM coryneine was inhibited reversibly by pretreatment with 5 μM pancuronium (Fig. 4B). Pancuronium at concentrations of 1 and 5 μM inhibited the coryneine (0.2 mM)-induced depolarization reducing it to 77.2 ± 9.9% (n = 4) and 32.4 ± 4.3% (n = 4) of the control value, respectively.

The amount of depolarizations induced by coryneine (20 μM—2 mM), succinylcholine (1 μM—1 mM) and ACh (1 μM—1 mM) was compared between normal endplate regions and denervated muscle membrane (Fig. 5A and 5B). ACh-induced depolarization, but not coryneine- and succinylcholine-induced depolarization, exhibited hypersensitivity in the denervated muscle.

Coryneine-Induced Contraction and Its Inhibition by Pancuronium in Denervated Muscles Coryneine (30 μM—10 mM) produced contractions of denervated muscles in a concentration-dependent manner. The contracting effect of coryneine was reduced in the presence of 70 mM pancuronium (Fig. 6A). In the double reciprocal plot, straight lines were obtained by assuming a Hill coefficient (nH) of 1.5 (Fig. 6B). The values of the estimated maximal response and 50% effective concentration (EC50) of coryneine in the presence and absence of pancuronium were 41.1% (37.0—46.2%; 95% confidence limits), 0.891 mM (0.666—1.51 mM) and 53.7% (47.1—62.6%), 0.308 mM (0.275—0.352 mM), respectively. Pancuronium increased the EC50 value of coryneine on decreasing the maximal response.

DISCUSSION

Coryneine blocked the neuromuscular transmission and depolarized the endplate region like succinylcholine and carbamylcholine. Cuthbert supposes the action of coryneine to be a depolarizing blockade because the neuromuscular blocking effect of coryneine was not reversed by neostigmine in a cat sciatic nerve-tibial muscle preparation in situ. The neuromuscular blocking effect of coryneine in our experiment, however, was reversed by neostigmine. In addition, the electrical charge—response curve of the ACh potential in the presence and absence of coryneine showed that its blocking action was a mixture of competitive and noncompetitive inhibition. This difference in blocking behavior may be caused by differences in the preparation and animals used. In some species, succinylcholine also initially exhibited a depolarizing action but the action became a competitive one during the blocking process (namely a dual block). In the present experiment, the neuromuscular blockade induced by succinylcholine was not reversed by neo-
Fig. 5. Comparison of the Depolarizing Effects Produced by Corynine (○), Acetylcholine (△) and Succinylcholine (□) between Isolated Normal (A) and Denervated (B) Mouse Diaphragm Muscle Cells

Depolarizations from resting membrane potential are plotted against the log concentration of each drug. Note that only ACh sensitivity was higher in denervated muscle cells than normal ones. The depolarization caused by high concentrations of drugs was not recorded in denervated muscle because of the marked contraction. The resting membrane potentials of normal and denervated muscle cells were $-67.0 \pm 0.5$ mV (mean ± S.E.M.; $n=176$ cells/47 muscles) and $-59.3 \pm 0.2$ mV (mean ± S.E.M.; $n=168$ cells/30 muscles), respectively. Values are mean ± S.E.M. ($n=13-27$ cells/4-6 muscles, 1-9 cells/muscle).

Fig. 6. Corynine-Induced Contraction and Its Inhibition by Pancuronium in Denervated Mouse Diaphragm Muscles

A: log concentration–contraction curves of corynine in the absence (○) and presence (●) of 70 nM pancuronium are shown. The corynine-induced contractions were estimated as a percentage of the contraction for 1.65 mM acetylcholine. Pancuronium was applied 10 min before application of corynine. B: The double-reciprocal plots were constructed from data in A. The straight lines are obtained by least-square fitting of the data assuming a Hill coefficient ($n_H$) of 1.5. Values are means ± S.E.M. ($n=4$ muscles).

stigmine, even if succinylcholine was applied for 1 h. Therefore, succinylcholine did not exhibit dual blockade in the mouse diaphragm muscle preparation. Corynine may cause neuromuscular blockade by a dual mode of action in that preparation.

Corynine itself at concentrations above 20 μM exhibited a depolarizing action which was antagonized by pancuronium. The amount of depolarization produced by corynine and succinylcholine was not enhanced in denervated muscles although ACh exhibited hypersensitivity. Cholinesterase activity was reported to fall in denervated muscle.\(^{14-20}\) This fact, however, does not affect the result that only ACh exhibited hypersensitivity, because Lorkovic and Rubel\(^{11}\) have reported that the amount of depolarization produced by ACh was not altered by pretreatment with cholinesterase inhibitor in normal muscles when using the superfusion technique that we ourselves used. These results indicate that the depolarizing action of corynine is different from that of ACh.

Corynine is a quaternary ammonium derivative of dopamine and we have observed that dopamine did not cause neuromuscular block. Catecholamines have been reported to contract isolated chronically denervated muscles\(^{21-23}\) and to exhibit a neuromuscular blocking action.\(^{22,24}\) The catecholamine-induced contraction of denervated muscle is, however, unaffected by d-tubocurarine.\(^{21}\) In this study, we showed that the corynine-induced contraction of denervated muscle was reduced by pancuronium. The blocking action was also shown to be a mixed type of competitive and noncompetitive antagonism, like the blocking action of corynine on
ACh potential. The effects of coryneine obviously differ from those of catecholamines.

In conclusion, coryneine is a depolarizing and mixed-type competitive and noncompetitive neuromuscular blocker in an isolated mouse diaphragm muscle preparation.

Acknowledgments We are grateful to Prof. T. Koizumi (Department of Synthetic Organic Chemistry, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University) and Dr. T. Uwano for the synthesis of coryneine.

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