# Interactions between ORG9426 and Other Non-depolarizing Neuromuscular Blocking Agents in Rats *In Vivo*

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In this study, combined neuromuscular blocking effects of ORG9426 with other non-depolarizing neuromuscular blocking agents were investigated. About 20% steady state neuromuscular block was established by a continuous infusion of one of 6 neuromuscular blocking agents (ORG9426, vecuronium, pancuronium, pipecuronium, d-tubocurarine and metocurine). Then 1/7 of the ED50 of ORG9426 or one of other neuromuscular blocking agents was administered in a single injection, and the increase in the neuromuscular block was observed. The combined neuromuscular blocking effect of ORG9426 and d-tubocurarine or ORG9426 and metocurine was significantly ($P < 0.05$) greater than that of each corresponding control (the combination of same neuromuscular blocking agent). The effect of d-tubocurarine was also potentiated by vecuronium, pancuronium and pipecuronium. These potentiations were not observed between ORG9426 and pancuronium, pipecuronium or vecuronium. Possible mechanisms of these synergistic interactions were discussed. (Key words: neuromuscular transmission, neuromuscular blocking agent, ORG9426, drug interaction)

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ORG9426 is a new non-depolarizing neuromuscular blocking agent (NMBA), which is the 3-hydroxy-2-morpholino-16-allyl-pyrrolidine derivative of vecuronium (fig. 1). It was reported that the duration of action of ORG9426 is similar to that of vecuronium, but the onset of action is considerably faster than that of vecuronium or pancuronium in man[1].

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It has been reported that in *in vitro* experiments[2–5], and in clinical studies[6–11], interaction of two different non-depolarizing NMBA can be more than additive.

In the present study, the interactions of ORG9426 with other non-depolarizing NMBA (d-tubocurarine, metocurine, pancuronium, pipecuronium and vecuronium) were investigated in rats *in vivo*.

## Methods

All experiments were performed on anesthetized male Sprague-Dawley rats weighing between 300–350g. Anesthesia was induced by intraperi-

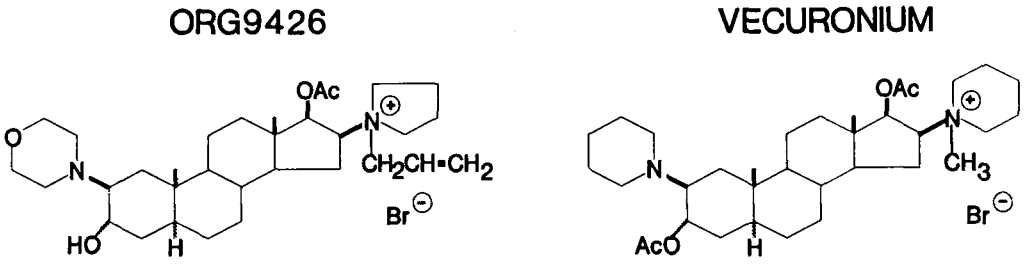


**Fig. 1.** Chemical structure of ORG9426 and vecuronium.

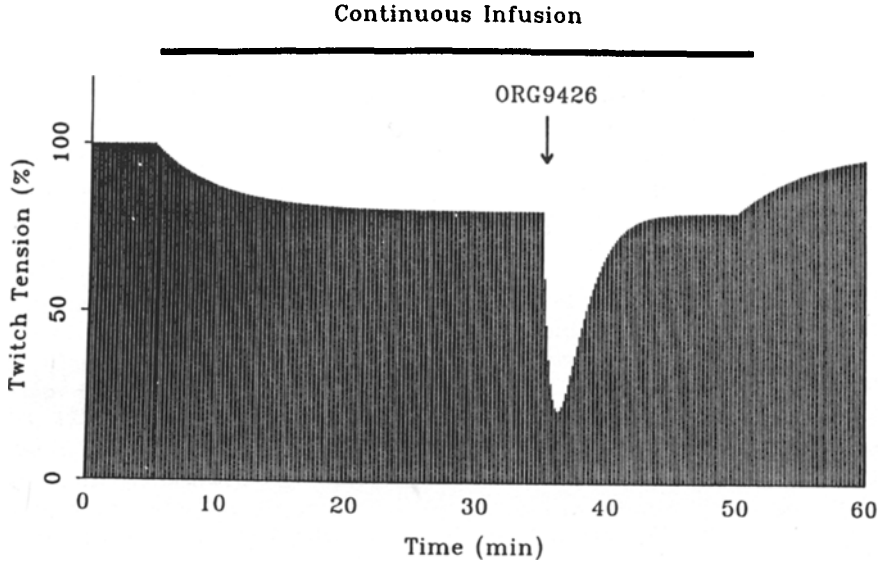


**Fig. 2.** Schematic representation of experiments performed.
After a steady state, about 20%, neuromuscular block was produced by continuous infusion of one of the NMBA, one seventh of the ED50 of ORG9426 or one of the other NMBA was administered in a single injection. Resulting decrease in the force of contraction was observed.

toneal administration of a mixture of 40 mg·kg$^{-1}$ pentobarbital and 500 mg·kg$^{-1}$ urethane. Animals were ventilated with oxygen via tracheostomy with a Harvard Rodent Ventilator at the rate of 60 per minute with a tidal volume of 1 ml per 100g body weight. The common carotid artery and both external jugular veins were cannulated for monitoring of arterial pressure and administration of drugs, respectively. Rectal temperature was maintained at 36.5–37.5°C using a heating lamp. Bipolar platinum electrodes were placed on the sciatic nerves at the gluteal region. The nerves were crushed with a ligature proximal to the electrodes. The distal tendon of tibialis anterior muscle was dissected and connected by a light steel wire to a force displacement transducer (Grass, FT-03). The nerve was stimulated with supramaximal square wave stimuli of 0.2 ms duration at 0.1 Hz with a stimulator (Grass, S88). The force of contraction of the tibialis anterior muscle was continuously recorded on a polygraph (Grass, 7D).

Table 1. Effect of ORG9426

| Partial Block by | Twitch Tension (% of control) | | | P |
|---|---|---|---|---|
| | Before* | After* | Changes | |
| ORG9426 | 78.4 ± 0.7 | 19.9 ± 4.5 | 58.4 ± 4.3 | control |
| Vecuronium | 77.0 ± 1.0 | 24.2 ± 5.8 | 52.7 ± 5.4 | N.S. |
| Pancuronium | 76.0 ± 0.9 | 18.4 ± 3.9 | 56.8 ± 3.8 | N.S. |
| Pipecuronium | 76.2 ± 1.5 | 8.7 ± 2.9 | 67.4 ± 3.0 | N.S. |
| d-Tubocurarine | 77.5 ± 1.0 | 5.1 ± 2.4** | 72.4 ± 2.9 | <0.05 |
| Metocurine | 79.2 ± 0.8 | 2.8 ± 2.8*** | 76.4 ± 2.4 | <0.05 |

All values represent mean ± SEM of 6 experiments.
*Before and after a single injection of 100 $\mu g \cdot kg^{-1}$ of ORG9426.
**Complete block in 2 experiments.
***Complete block in 5 experiments.

NMBA used were ORG9426, vecuronium bromide, pancuronium bromide, pipecuronium bromide (gift from Organon, Inc.), d-tubocurarine chloride (Sigma Co.), and metocurine iodide (gift from Eli Lilly Co.).

After an initial stabilization period, a steady state, about 20%, neuromuscular block was produced by the continuous infusion of one of the 6 NMBA. Then, one seventh of the ED50 (dose required to produce a 50% depression of twitch height) of ORG9426 or one of the other NMBA was injected into the contralateral jugular vein in a single injection, and the combined neuromuscular effect was observed (fig. 2). Actual doses used were; ORG9426 100 $\mu g \cdot kg^{-1}$, vecuronium 36.1 $\mu g \cdot kg^{-1}$, pancuronium 12.0 $\mu g \cdot kg^{-1}$, pipecuronium 5.7 $\mu g \cdot kg^{-1}$, d-tubocurarine 3.7 $\mu g \cdot kg^{-1}$, metocurine 1.6 $\mu g \cdot kg^{-1}$.

Results were analyzed using one way analysis of variance followed by Duncan's multiple range test. Combinations of same NMBA were used as control. $P$ value less than 0.05 was considered as significant. All values are expressed as mean ± SEM of six experiments.

## Results

The effects of 1/7 of ED50 of ORG9426 in the presence of the partial neuromuscular block are summarized in table 1. When the partial neuromuscular block was produced by a continuous infusion of ORG9426, a single injection of ORG9426 decreased the force of contraction by 58.4 ± 4.3% (from 78.4 ± 0.7% to 19.9 ± 4.5%). This served as control. In the presence of the partial neuromuscular block produced by d-tubocurarine or metocurine, the decrease in the force of contraction was 72.4 ± 2.9% (from 77.5 ± 1.0% to 5.1 ± 2.4%) or 76.4 ± 2.4% (from 79.2 ± 0.8% to 2.8 ± 2.8%), respectively. These are both significantly different from control ($P < 0.05$). The effect of ORG9426 was not different from control in the presence of partial neuromuscular block produced by vecuronium, pancuronium or pipecuronium.

In the presence of the partial neuromuscular block produced by ORG9426, the effect of 1/7 of the ED50 of d-tubocurarine was significantly greater than that of control (table 2. $P < 0.01$). The effect of d-tubocurarine was also potentiated by the infusion of vecuronium, pancuronium and pipecuronium, but not by metocurine.

The effect of 1/7 of the ED50 of metocurine was potentiated by ORG9426 ($P < 0.05$), but not by other

**Table 2.** Effect of d-Tubocurarine

| Partial Block by | Twitch Tension (% of control) | | | *P* |
|---|---|---|---|---|
| | Before* | After* | Changes | |
| d-Tubocurarine | 76.5 ± 0.30 | 60.2 ± 2.6 | 17.4 ± 2.2 | control |
| ORG9426 | 77.3 ± 1.0 | 38.3 ± 3.2 | 39.0 ± 3.1 | <0.01 |
| Vecuronium | 79.2 ± 0.9 | 43.6 ± 3.9 | 35.6 ± 3.3 | <0.01 |
| Pancuronium | 79.5 ± 0.5 | 41.1 ± 3.2 | 38.3 ± 2.8 | <0.01 |
| Pipecuronium | 78.1 ± 0.7 | 45.7 ± 2.7 | 32.3 ± 3.3 | <0.01 |
| Metocurine | 79.7 ± 0.3 | 57.2 ± 2.9 | 22.5 ± 2.8 | N.S. |

All values represent mean ± SEM of 6 experiments.
*Before and after a single injection of 3.7 $\mu g \cdot kg^{-1}$ of d-tubocurarine.

**Table 3.** Effect of metocurine

| Partial Block by | Twitch Tension (% of control) | | | *P* |
|---|---|---|---|---|
| | Before* | After* | Changes | |
| Metocurine | 77.3 ± 0.9 | 39.7 ± 2.9 | 37.7 ± 2.6 | control |
| ORG9426 | 74.4 ± 0.7 | 21.6 ± 3.3 | 52.8 ± 3.0 | <0.05 |
| Vecuronium | 79.8 ± 0.5 | 49.7 ± 4.0 | 30.1 ± 4.0 | N.S. |
| Pancuronium | 78.8 ± 0.7 | 31.1 ± 1.6 | 47.8 ± 1.6 | N.S. |
| Pipecuronium | 77.2 ± 1.0 | 31.0 ± 5.1 | 46.2 ± 5.0 | N.S. |
| d-Tubocurarine | 75.8 ± 1.4 | 33.1 ± 3.7 | 42.7 ± 4.3 | N.S. |

All values represent mean ± SEM of 6 experiments.
*Before and after a single injection of 1.6 $\mu g \cdot kg^{-1}$ of metocurine.

NMBA (table 3).

The partial neuromuscular block by ORG9426 did not potentiate the effect of vecuronium, pancuronium and pipecuronium (table 4, 5 and 6).

## Discussion

There are several reports concerning the neuromuscular blocking effects of various combinations of non-depolarizing NMBA. For example, the combination of alcuronium with pancuronium[4,7], pancuronium with vecuronium[8,9] and tubocurarine with metocurine[6] were reported to give the simple additive effect. On the other hand, the combination of d-tubocurarine with pancuronium[2,4,6], metocurine with pancuronium[2,6], d-tubocurarine with vecuronium[2,10] and atracurium with pancuronium[11] were reported to give the more than additive effect. Golpariani et al. have reported that the marked potentiation of neuromuscular blocking effect was observed between ORG9426 and d-tubocurarine in rat *in vitro*[3].

In this study, the mutual potentiation of the neuromuscular blocking effect was observed between ORG9426 and d-tubocurarine and between ORG9426 and metocurine, with disregard to their order of administration. However, no potentiation was observed on the neuromuscular blocking effect by the combination of ORG9426 with vecuronium, pancuronium or pipecuronium. It was also found that the effect of d-tubocurarine was potentiated by pancuronium, vecuronium and pipecuronium, but not by metocurine.

**Table 4.** Effect of vecuronium

| Partial Block by | Twitch Tension (% of control) | | | P |
|---|---|---|---|---|
| | Before* | After* | Changes | |
| Vecuronium | 76.7 ± 1.2 | 34.1 ± 4.8 | 42.4 ± 4.4 | control |
| ORG9426 | 75.7 ± 0.9 | 38.0 ± 5.8 | 37.5 ± 5.8 | N.S. |

All values represent Mean ± SEM of 6 experiments.
*Before and after a single injection of 36.1 $\mu g \cdot kg^{-1}$ of vecuronium.

**Table 5.** Effect of pancuronium

| Partial Block by | Twitch Tension (% of control) | | | P |
|---|---|---|---|---|
| | Before* | After* | Changes | |
| Pancuronium | 76.3 ± 0.9 | 52.5 ± 3.2 | 23.8 ± 2.8 | control |
| ORG9426 | 76.7 ± 0.8 | 47.5 ± 3.2 | 29.1 ± 2.6 | N.S. |

All values represent mean ± SEM of 6 experiments.
*Before and after a single injection of 12.0 $\mu g \cdot kg^{-1}$ of pancuronium.

**Table 6.** Effect of pipecuronium

| Partial Block by | Twitch Tension (% of control) | | | P |
|---|---|---|---|---|
| | Before* | After* | Changes | |
| Pipecuronium | 76.8 ± 1.1 | 52.9 ± 3.1 | 23.8 ± 3.1 | control |
| ORG9426 | 77.2 ± 1.0 | 56.6 ± 3.7 | 20.4 ± 3.3 | N.S. |

All values represent mean ± SEM of 6 experiments.
*Before and after a single injection of 5.7 $\mu g \cdot kg^{-1}$ of pipecuronium.

Most NMBA can be divided into two groups according to their chemical structure, namely aminosteroid and bisbenzyltetrahydroisoquinoline groups. ORG9426, pancuronium, vecuronium and pipecuronium belong to the former, while d-tubocurarine and metocurine belong to the latter group. Bowman suggested that pairs within the same group exhibited simple addition, whereas pairs comprising one from each group exhibited potentiation[12]. Our results and many other reports are in accordance with this hypothesis.

There was yet no conclusive explanation for the potentiation between different nondepolarizing NMBA. We can only speculate about the mechanism underlying the present findings. It is conceivable that these potentiation is caused by the greater inhibition by some NMBA of the pre-synaptic nicotinic positive feed back mechanism of the evoked release of acetylcholine at the neuromuscular junction[13]. If the two drugs that act at two sites to relatively different degrees are used in combination, both of these actions might simultaneously occur to a greater extent than when either drug is used alone[6]. It has been suggested that vecuronium, pancuronium and alcuronium are predom-

inantly post-synaptic blockers, while d-tubocuraine has both pre- and post-synaptic effect[4,13]. Because of its structural similarity, ORG9426 probably behaves similarly to other aminosteroidal NMBA. The potentiation observed between d-tubocurarine and ORG9426 or other aminosteroidal NMBA might be due to the differences in the affinity of these compounds to pre- and postsynaptic nicotinic receptors.

In addition to aforementioned mechanism of potentiation, Waud and Waud reported that this potentiation could also be observed even in the experiments which eliminated the pre-synaptic component by the use of externally administered agonist (carbachol)[14]. This findings suggested the involvement of some other mechanisms of the potentiation. Namely, the postsynaptic nicotinic acetylcholine receptor is composed of five protein subunits and two of them, designated $\alpha$, offer the binding sites for acetylcholine. These two $\alpha$ subunits have identical amino acid sequences, but because of the differences in neighboring subunits, they do not exhibit equal affinity to the various NMBA[15,16]. If it would be supposed that aminosteroidal group of NMBA preferentially combines with the one of the two $\alpha$ subunits while the bisbenzyltetrahydroisoquinoline group (d-tubocurarine type) of NMBA preferentially combine with the other, it is conceivable that the differences of the affinity to two binding sites among NMBA may be responsible for the synergistic effect observed in some combinations of different NMBA[14].

Regardless of the mechanism, this potentiation of neuromuscular blocking effect of ORG9426 with d-tubocurarine or metocurine, if confirmed in man, may have clinical significance. It was reported that the onset time of ORG9426 was shorter than those of other non-depolarizing NMBA[1]. Administration of ORG9426 after a priming dose of metocurine or d-tubocurarine might provide superior intubating conditions.

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