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PARADOXICAL ANTAGONISM OF NEUROMUSCULAR BLOCK BY VECURONIUM METABOLITES

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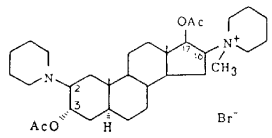
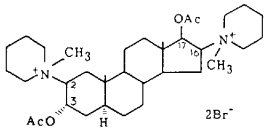
Abstract. Vecuronium is hydrolyzed in the body to 3-deacetyl (ORG 7268), 17-deacetyl (ORG NC58), and 3, 17-bis-deacetyl (ORG 7402) derivatives. Interactions of vecuronium and these metabolites were studied in phrenic nerve - hemidiaphragm preparations of rats. As already reported, ORG 7268 had a potent neuromuscular blocking action, and ORG NC58 and ORG 7402 had a weak neuromuscular blocking action. As expected, ORG 7268 increased the degree of neuromuscular block by vecuronium. However, a low concentration (10 μM) of ORG NC58 and ORG 7402 *reversed* the block by vecuronium. At a high concentration (50 μM), ORG NC58 and ORG 7402 increased the degree of block by vecuronium. Although we do not have enough data to explain these paradoxical reversal of neuromuscular block at this moment, we postulate that these results reflect the interaction between "slow" and "fast" competitive antagonists. Regardless of the mechanism, it should be emphasized that the concentrations of ORG NC58 and ORG 7402 which are necessary to reverse the block are much lower than those which facilitate the block. It is conceivable that this paradoxical reversal of the block occurs in experimental and clinical situations. Therefore, in determining the neuromuscular blocking action of a compound, the "antagonistic" effect of its metabolites should also be considered.

Key words : vecuronium, neuromuscular transmission, drug interaction, competitive inhibition, muscle relaxant.

Vecuronium bromide, 1-[3, 17-bis(acetyloxy)-2-(1-piperidinyl)androstan-16yl]-1-methylpiperidinium bromide, is a non-depolarizing neuromuscular blocking agent and monoquaternary homologue of pancuronium bromide (Fig. 1). As compared with pancuronium, vecuronium has several clinical advantages, *e.g.*, shorter duration of action and no cardiovascular side effects (1). Vecuronium is relatively unstable at physiological pH 7.4, and undergoes deacetylation in the 3 and 17 positions (2). It is probable that vecuronium is firstly hydrolyzed to its 3-deacetyl from, ORG 7268 (Fig. 2). Hydrolysis also takes place to form 17-deacetyl derivative (ORG NC58), and finally 3, 17-bis-deacetyl derivative (ORG 7402). It is already reported that these metabolites have neuromuscular blocking activity themselves (3, 4). The aim of this study was to examine the interaction of these compounds with vecuronium on neuromuscular transmission.



Vecuronium bromide (ORG NC45)



Pancuronium bromide

Fig. 1. Chemical structures of vecuronium bromide (ORG NC 45) and pancuronium bromide. Vecuronium is a 16-monoquaternary homologue of pancuronium.

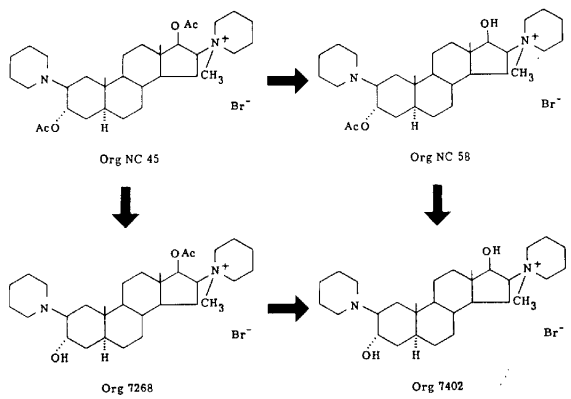


Fig. 2. Chemical structures of vecuronium and its metabolites. Vecuronium undergoes deacetylation in the 3 and 17 positions, forming 3-deacetyl (ORG 7268), 17-deacetyl (ORG NC58) and 3, 17-bis-deacetyl derivatives (ORG 7402).

MATERIALS AND METHODS

The experiments were carried out on phrenic nerve - hemidiaphragm preparations of rats (5). Male Sprague-Dawley rats of 250-300 g body weight were decapitated and the hemidiaphragms were dissected with their phrenic nerves. The preparations were then placed in double walled glass organ baths filled with modified Krebs' solution, containing 1.4 mM Ca^{++} and 0.9 mM Mg^{++}, which are the same as normal ionized Ca^{++} and Mg^{++} concentrations, respectively (6). The solution was kept at 37℃ and aerated with 95 % oxygen - 5 % carbon dioxide. Phrenic nerves were stimulated through bipolar platinum electrodes with supramaximal, 0.2-msec duration, 0.1-Hz square wave stimulation. "Train-of-four" stimulation, a short train of four stimuli at a frequency of 2 Hz, was also used occasionally (7). The indirectly elicited twitch tension was continuously monitored with a TB-611T force displacement transducer and recorded on an RM-6000 polygraph (Nihon Kohden Co.). After the twitch tension became stabilized, two series of experiments were performed: *Experiment A.* One of the vecuronium metabolites was given, and its own effects on the neuromuscular transmission

was examined. Experiment B. Partial neuromuscular block was induced with vecuronium (6 μM), then one of the vecuronium metabolites was added to the bath, and their combined effects on the neuromuscular transmission was examined. Besides these vecuronium metabolites, hexamethonium was also used in the two series of experiments as an example of a weak neuromuscular blocking agent.

The chemical compounds used were: vecuronium bromide, ORG 7268, ORG NC58, ORG 7402 (Gift of Japan Organon Co., Tokyo, Japan); hexamethonium bromide (Sigma Chemical Co., St. Louis, Mo. USA); neostigmine bromide (Wako Pure Chemical Industries, Osaka, Japan). The chemicals used for the preparation of the Krebs' solution were reagent grade or better.

Statistical analysis was performed using a paired Student's t test, where $p<0.05$ was considered significant.

RESULTS

All results are expressed as the twitch tension (TT, % of control), instead of using the degree of block. The results of the two series of experiments are summarized in Tables 1 and 2.

TABLE 1. NEUROMUSCULAR BLOCKING ACTION OF VECURONIUM METABOLITES AND HEXAMETHONIUM

	ORG NC58			neostigmine	
	10 μM	50 μM		100 nM	1 μM
Experiment 1		25 min	70 min		
	100.3±1.2	55.1±5.4	38.3±5.1	−	10.8±3.5*
	ORG 7402			neostigmine	
	10 μM	100 μM		100 nM	1 μM
Experiment 2		25 min	70 min		
	101.7±0.5	68.4±4.6	43.3±7.3	46.4±7.3*	31.5±7.8**
		hexamethonium 4 mM		neostigmine	
Experiment 3		10 min	40 min	100 nM	1 μM
		31.1	8.9	−	24.4

ORG NC58, ORG 7402 and hexamethonium induced gradually increasing neuromuscular block. So instead of calculating ED_{50} value, the twitch tension at two points (25 and 70 min for ORG NC58 and ORG 7402, 10 and 40 min for hexamethonium) is indicated in this table. Effects of ORG NC58 and ORG 7402 at a lower concentration (10 μM) were also examined prior to using higher concentrations. All results are expressed with the twitch tension, % of control (mean ± S.E.M., n=4). Following the production of neuromuscular block by ORG NC58, ORG 7402 and hexamethonium, neostigmine was added to the bath and its effects on the neuromuscular block were examined. Significant difference (paired t-test) from corresponding neuromuscular block by ORG NC58 (50 μM, 70 min) or ORG 7402 (100 μM, 70 min) : * $p<0.005$, ** $p<0.001$.

TABLE 2. ANTAGONISTIC EFFECTS OF VECURONIUM METABOLITES AND HEXAMETHONIUM ON THE NEUROMUSCULAR BLOCK BY VECURONIUM

	vecuronium	ORG 7268	
Experiment 1	6 μM	1 μM	5 μM
	30.4±9.4	18.0±7.4**	2.4±1.5
	vecuronium	ORG NC58	
Experiment 2	6 μM	10 μM	50 μM
	21.9±4.4	29.5±6.1*	0
	vecuronium	ORG 7402	
Experiment 3	6 μM	10 μM	50 μM
	24.1±6.4	41.5±6.6***	0
	vecuronium	hexamethonium	
Experiment 4	6 μM	400 μM	2 mM
	26.2±3.5	69.5±1.2****	13.2±3.7

In each experiment, 6 μM of vecuronium was given and the partial neuromuscular block was induced. Then one of vecuronium metabolites or hexamethonium was added and its effect on the neuromuscular block was examined. All results are expressed with the twitch tension, % of control (mean ± S.E.M., n = 4). Significant difference (paired *t*-test) from corresponding neuromuscular block by vecuronium alone: * $p<0.05$, ** $p<0.01$, *** $p<0.005$, **** $p<0.001$.

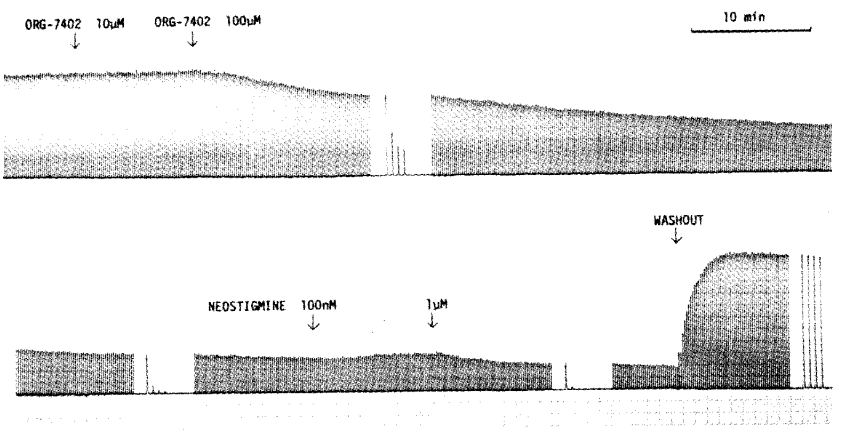


Fig. 3. Neuromuscular blocking action of ORG 7402. 10 μM of ORG 7402 showed no significant effect on the single twitch response. 100 μM of ORG 7402 induced gradually increasing neuromuscular block. The train-of-four ratio decreased. 100 nM of neostigmine slightly reversed the block, but 1 μM of neostigmine further increased the block. The twitch response quickly came back to the control level after washing the compounds out.

Experiment A. ORG 7268 had potent neuromuscular blocking activity. Neuromuscular block was produced by 5 μM and 7 μM of ORG 7268 (n = 6), then from the log dose-% response regression line from these data the ED_{50} value was calculated. The ED_{50} of ORG 7268 was 6.25 μM, which is about 1.3 times greater than that of vecuronium. ORG NC58 and ORG 7402 had relatively weak neuromuscular blocking activity. With 10 μM of ORG NC58 or ORG 7402, no significant change in TT was observed. At a higher concentration, the block increased gradually and never became stable (Fig. 3), so it was not possible to calculate ED_{50} values. With 50 μM of ORG NC58, TT decreased to 55.1 ± 5.4 % (mean ± S.E.M., n = 4) in 25 min, and 38.3 ± 5.1 % in 70 min. With 100 μM of ORG 7402, TT decreased to 68.4 ± 4.6 % (n = 4) in 25 min, and 43.3 ± 7.3 % in 70 min. The train-of-four ratio also decreased (48.6 ± 5.8 % in 15 min, and 6.8 ± 2.2 % in 60 min). At 100 nM, neostigmine slightly increased TT (43.3 ± 7.3 % to 46.4 ± 7.3, $p<0.001$). Hexamethonium also induced a gradually increasing neuromuscular block. With 4 mM of hexamethonium, TT decreased to 31.1 % in 10 min and 8.9 % in 40 min (Fig. 4). TT quickly came back to the control after washing out the compounds.

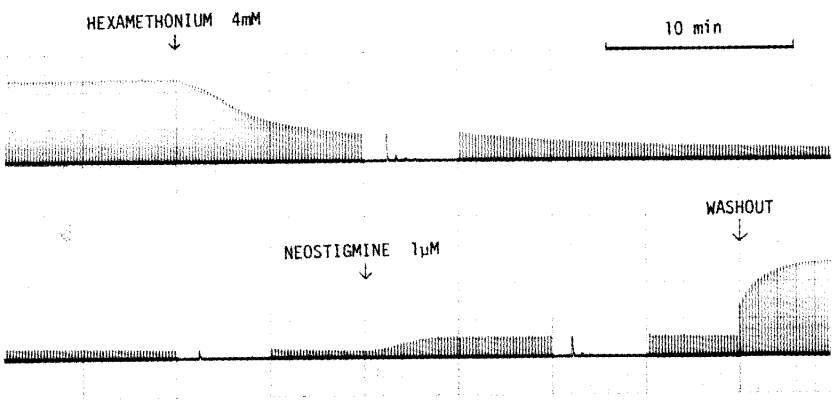


Fig. 4. Neuromuscular blocking action of hexamethonium. 4 mM of hexamethonium induced gradually increasing neuromuscular block. 1 μM of neostigmine partially reversed the block.

Experiment B. In the experiments with ORG 7268, 6 μM of vecuronium decreased TT to 30.4 ± 9.4 % (n = 4), and addition of 1 μM ORG 7268 further decreased TT to 18.0 ± 7.4 % ($p<0.01$). In this case, the two compounds seemed to be acting additively. In the experiments with ORG NC58, 6 μM vecuronium decreased TT to 21.9 ± 4.4 % (n = 4), and addition of 10 μM ORG NC58 significantly increased TT to 29.5 ± 6.1 % ($p<0.05$). However, addition of 50 μM ORG NC58 had decreased TT. In the experiments with ORG 7402, 6 μM vecuronium decreased TT to 24.1 ± 6.4 % (n = 4). Addition of 10 μM ORG 7402 significantly increased TT to 41.5 ± 6.6 % ($p<0.005$), but addition of 50 μM ORG 7402

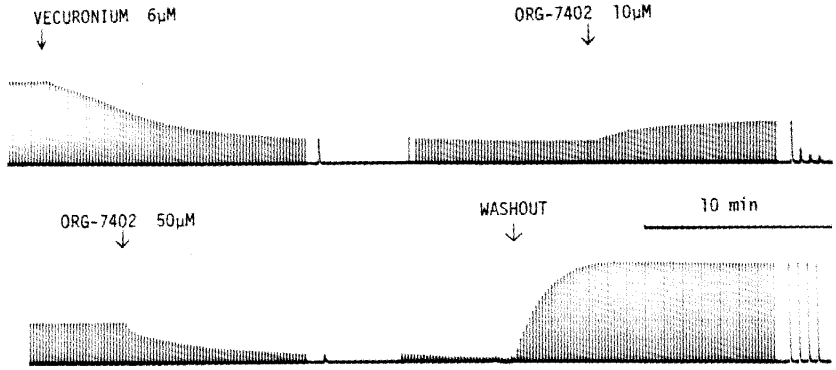


Fig. 5. Antagonism of neuromuscular block by ORG 7402. Partial neuromuscular block was induced by 6 μM of vecuronium, then ORG 7402 was added. 10 μM of ORG 7402 partially reversed the block, but 50 μM of ORG 7402 further increased the block.

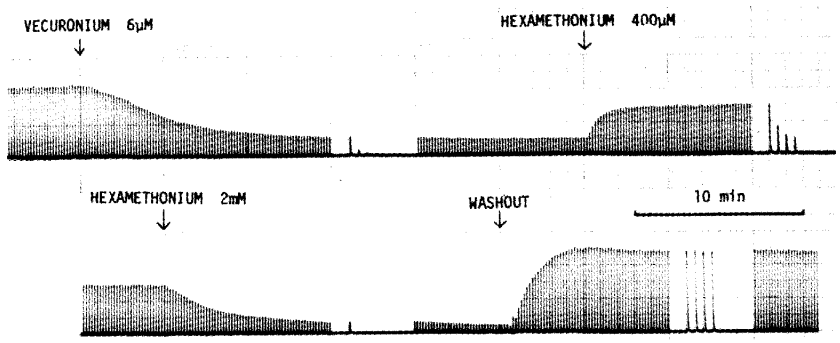


Fig. 6. Antagonism of neuromuscular block by hexamethonium. A low concentration of hexamethonium reversed the block, but a high concentration of hexamethonium increased the block by vecuronium.

decreased TT (Fig. 5). In the experiments with hexamethonium, 6 μM vecuronium decreased TT to 26.2 ± 3.5 % ($n = 4$), and addition of 0.4 mM hexamethonium increased TT to 69.5 ± 1.2 % ($p < 0.001$). However, addition of 2 mM hexamethonium decreased TT (Fig. 6).

ORG NC58, ORG 7402 and hexamethonium reversed the neuromuscular block by vecuronium at low concentrations, while these compounds alone had no significant effect on neuromuscular transmission at such low concentrations. At higher concentrations, these compounds seemed to act additively with vecuronium and increased the block.

DISCUSSION

Since the introduction of d-tubocurarine to the practice of anesthesia in 1942 (8), many muscle relaxants have been developed and tested in experimental animals, and a few of them have been used clinically. In 1971, Karis and Gissen described

ideal characteristics of neuromuscular blocking agents as follows (9): [a] The agent should be competitive and nondepolarizing. [b] The cessation of the block should not depend on excretion by kidney or liver, since known or unknown disease conditions could markedly prolong the action of such an agent. [c] Chemical decomposition of the drug in the body would be acceptable provided the breakdown products did not possess neuromuscular blocking power or produce other unwanted effects. [d] The relaxant should have a relatively short duration of action. [e] The action should be highly specific, so that no harmful effects on other systems would occur. [f] Block by a competitive agent would probably be reversible by an anticholinesterase.

Vecuronium is a nondepolarizing neuromuscular blocking agent with relatively short duration of action and no known side effects and is easily reversible by anticholinesterases. The cessation of the block depends on excretion by both kidney and liver, and chemical decomposition. Vecuronium undergoes deacetylation in the 3 and 17 positions, to form ORG 7268, ORG NC58 and ORG 7402. Although neuromuscular blocking activity of these metabolites is known, it is generally believed that they do not affect the block by vecuronium significantly, because ORG 7402, which is presumably the terminal metabolite of vecuronium, has very weak neuromuscular blocking activity, and it probably does not reach a high enough concentration to increase the block in a clinical situation (10).

If two compounds, A and B, are competitive antagonists of some agonist, it would seem self evident that addition of B where A is already present would increase the degree of block. In our experiments, though ORG 7268 increased the neuromuscular block by vecuronium, ORG NC 58 and ORG 7402 did not increase, but rather reversed the block at low concentrations. This paradoxical reversal of neuromuscular block is very interesting from the standpoints of both clinical relevance and mechanisms of reversal.

Clinical relevance. To maintain surgical muscle relaxation, it is necessary to keep the single twitch response at less than 25 % of the control (more than 75 % neuromuscular block). In clinical practice with vecuronium, 0.08 mg/kg of vecuronium is used as an initial dose, and it keeps the twitch response below 25 % for about 36 min. Then 0.02 mg/kg is used as a repeating dose, and this keeps the twitch response below 25 % for about 22 min under halothane anesthesia. From our study, the ED_{50} of vecuronium in man is 0.02, 0.016 and 0.014 mg/kg under NLA, halothane and enflurane anesthesia respectively (unpublished data). By simple calculation, it is easily estimated how much vecuronium should be used during anesthesia. For 2 h of surgical muscle relaxation under halothane anesthesia, 0.16 mg/kg of vecuronium is needed, and this amount is 10 times as much as the ED_{50} value. In our *in vitro* study, the ED_{50} of vecuronium was 4.8 μM, and 10 μM of ORG 7402 alone did not have any effect on the twitch response, while 100 μM of ORG 7402 caused neuromuscular block. In the experiments with vecuronium, 10 μM of ORG 7402 reversed the block by vecuronium, and 50 μM of ORG 7402

increased the block. These concentrations are about 2 times and 10 times as much as the ED_{50} value of vecuronium, respectively. Although only limited data are available about the pharmacokinetics of vecuronium metabolites (11), it is not likely that the terminal metabolite of vecuronium, ORG 7402, would be produced in sufficient quantities to increase the block by vecuronium, but it might accumulate enough to reverse the block by vecuronium. So, to interpret the pharmacokinetic and pharmacodynamic data of vecuronium, it is advisable to take into account this antagonistic effect of vecuronium metabolites.

Mechanisms of reversal. There are several possible explanations for this paradoxical reversal of neuromuscular block:

[a] "The metabolites, ORG NC58 and ORG 7402, are not competitive antagonists, but are depolarizing agents." Considering the characteristics of their parent compound and the chemical structures of these metabolites, it is unlikely that they possess depolarizing activity (12). It is probable that their neuromuscular blocking action is based on competitive antagonism and open channel blocking action (13).

[b] "They increase the release of acetylcholine from the nerve terminal." To examine this hypothesis, it is necessary to do electrophysiological studies of the motor endplate. At the neuromuscular junction, aminopyridines (2-, 3-, 4-aminopyridines, AP), diaminopyridines (2,3-, 2,6-, 3,4-diaminopyridines, DAP), tetraethylammonium (TEA) and guanidine are known to increase transmitter release evoked by nerve impulses. It is demonstrated that they enhance the influx of calcium ions during nerve terminal depolarization (14, 15). AP, DAP and TEA are also known to selectively block potassium conductance increases in nerve membranes (16, 17). It is suggested that AP, by prolonging the presynaptic action potential due to the decreased potassium conductance, increases the calcium concentration in the nerve terminal and, thus, the transmitter release (18). Lund and Thesleff suggested that AP, TEA and guanidine act directly on voltage-sensitive calcium channels in the nerve terminal membrane in such a way that the entry of calcium ions into the nerve terminal is facilitated (15). All these compounds can reverse the non-depolarizing neuromuscular block.

[c] "They have potent anti-acetylcholinesterase activity." It is reported that pancuronium and vecuronium have very weak anti-acetylcholinesterase activity (4). Our preliminary study showed that vecuronium metabolites are as potent as vecuronium and much weaker than pancuronium in terms of anti-butyrylcholinesterase activity.

[d] "The paradoxical reversal results from the interaction between slow and fast competitive antagonists." If two compounds are competitive antagonists of the same agonist, one would expect that addition of a second antagonist where the first antagonist is already present would increase the degree of block. However, in 1969, Stephenson and Ginsborg suggested that there is another possibility where the receptors are exposed to the agonist for only a short time (19). If the

antagonist dissociates from the receptors slowly, most of the receptors it occupies are not available to the agonist. When the fast antagonist is added to this system and equilibrated, fewer receptors are occupied by the slow antagonist. Although there are fewer receptors left free, the agonist can occupy more receptors than before, because the agonist will equilibrate with the fast antagonist. This paradoxical effect has been demonstrated experimentaly in the guinea-pig ileum and discussed quantitatively in detail (20).

At the neuromuscular junction, released acetylcholine molecules are hydrolyzed within a few milli-seconds by acetylcholinesterase (21). If one of two compounds is a slowly dissociating antagonist and the other a rapidly dissociating one compared with the acetylcholine life time at the neuromuscular junction, the paradoxical reversal of the block will be observed. In 1971, Ferry and Marshall reported an anticurare effect of hexamethonium in a phrenic nerve - hemidiaphragm preparation of the rat (22), and later concluded that this was the example of fast and slow antagonist interaction (23). Blackman *et al.* confirmed this effect and also reported the anticurare effect of gallamine and hyoscine butylbromide (24).

In our experiments, it was demonstrated that two vecuronium metabolites, ORG NC58 and ORG 7402, had weak neuromuscular blocking action and could reverse the neuromuscular block produced by vecuronium. These characteristics of vecuronium metabolites are similar to those of hexamethonium. Although we do not have enough data to reach a final conclusion at this moment, it seems likely that the antagonistic effect of these metabolites is due to the interaction of fast and slow antagonists.

Regardless of the mechanism, it should be emphasized that the concentrations of ORG NC58 and ORG 7402 which are necessary to reverse the block are much lower than those which facilitate the block. If the fourth hypothesis is applicable to the paradoxical reversal of the block, this phenomenon might occur with some other competitive antagonists of synaptic transmission. Therefore, in determining the action of a competitive antagonist, the antagonistic effect of its metabolites should also be considered.

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